(54) Title: ASSOCIATIONS OF POLYMORPHISMS IN THE FRZB GENE WITH OBESITY AND OSTEOPOROSIS

(57) Abstract: Methods and reagents for determining sequence variants present at the FRZB locus, which facilitate identifying individuals at risk for obesity and/or osteoporosis, are provided. In particular, methods for detecting an individual's risk for obesity and/or osteoporosis by detecting at least one polymorphism (e.g., at least one single nucleotide polymorphism) in the FRZB gene in a nucleic acid sample from the individual are provided. Related kits, arrays and systems are also described.

ASSOCIATIONS OF POLYMORPHISMS IN THE FRZB GENE WITH OBESITY AND OSTEOPOROSIS

5   FIELD OF THE INVENTION

The invention relates to methods and reagents for detecting an individual's risk for obesity and/or osteoporosis. More specifically, it relates to methods and reagents for detecting an individual's increased or decreased risk for obesity and/or osteoporosis by identifying the presence of at least one polymorphism in the FRZB gene.

10   BACKGROUND OF THE INVENTION

Transplantation experiments by Spemann and Mangold (1924, _Arch. Mikroskopische Anat. Entwicklungsmechanik_, 100:599-638) established the presence of an anatomically discrete region, the Spemann organizer, or dorsal lip, that controls patterning of the developing body axis in vertebrate embryos. Diffusible factors emanating from this
15   region were found to be involved in different developmental processes. The arrangement of Drosophila cuticle hairs in a defined polarity was found to be genetically controlled by 'frizzled' (FZD1), a 7-transmembrane receptor with a large extracellular cysteine-rich domain.

The bovine and human homologs of FZD1 were cloned by RT-PCR and by screening
20   bovine articular cartilage and human placenta cDNA libraries, which identified cDNAs encoding FRZB, the mammalian analog of FZD1 (Hoang _et al._, 1996, _J. Biol. Chem._, 271:26131-26137). The deduced 325-amino acid bovine and human FRZB proteins share 94% amino acid identity. Sequence analysis predicted that FRZB contains a 25-amino acid signal peptide, an N-terminal N-glycosylation site, a 24-amino acid putative
25   transmembrane segment, a region with multiple potential Ser/Thr phosphorylation sites, and a serine-rich C-terminal domain. The N-terminal region of FRZB shares 50% amino acid identity, including the conservation of 10 Cys residues, with frizzled. Immunoblot analysis determined that FRZB is expressed as an approximately 36-kD protein. In situ hybridization analysis of human embryos representing different stages
30   of development detected no expression from week 6 through week 13 except in the developing appendicular skeleton, as well as in several craniofacial bones and epiphyseal

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ends of the rib cage. Immunochemical analysis confirmed the expression of FRZB in the developing skeletal structures.

Northern blot analysis revealed that FRZB is expressed strongly in placenta and heart, at intermediate levels in brain, skeletal muscle, kidney, and pancreas, and at low levels in lung and liver (Leyns et al., 1997, Cell 88:747-756). SDS-PAGE analysis detected secretion of FRZB, possibly after proteolytic cleavage, consistent with FRZB's lack of the 7 transmembrane domains found in the Drosophila and vertebrate frizzled gene family. Functional analysis in Xenopus embryos showed that FRZB can antagonize the early and late effects of WNT8 signaling. Mammalian WNT genes include oncogenes that lead to mammary tumors. For further characterization of FRZB, see, e.g., Dann et al., 2001, Nature 412:86-90; Rattner et al., 1997, Proc. Nat. Acad. Sci. 94:2859-2863; and Schumann et al., 2000, Cardiovasc. Res. 45:720-728.

Leyns et al. (supra) mapped the human FRZB gene to 2q31-q33. They noted that loss of one copy of the 2q arm occurs with high incidence in lung and colorectal carcinomas, as well as in neuroblastomas, and suggested that FRZB might function as a tumor suppressor gene. Hoang et al. (supra) suggested FRZB might play a role in skeletal morphogenesis. However, a direct role for the FRZB gene in human disease and development has not been identified.

Among other aspects, the present invention provides alleles of FRZB, identified by the presence of one or more predisposing or protective polymorphisms, that are associated with an increased or decreased risk for obesity and/or osteoporosis. A complete understanding of the invention will be obtained upon review of the following.

SUMMARY OF THE INVENTION

The invention provides methods, reagents and kits for detecting an individual's increased or decreased risk for obesity and/or osteoporotic diseases and related diseases. In certain embodiments, the methods are used to determine an individual's risk for obesity and related diseases. In other embodiments, the methods are used to determine an individual's risk for osteoporosis and related diseases. In further embodiments, the methods of determining risk are combined with known clinical methods to diagnose osteoporosis or obesity.

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A first general class of embodiments provides methods for determining an individual's risk for obesity. In the methods, presence of at least one obesity-related polymorphism in a frizzled-related protein (FRZB) gene in a nucleic acid sample of the individual is detected. The presence of the at least one polymorphism provides an indication of the
5   individual's risk for obesity. The individual's risk for obesity can be, e.g., either an increased risk or a decreased risk as compared to an individual without the at least one polymorphism (e.g., an individual with a different allele at that polymorphic site). Accordingly, the at least one polymorphism can comprise a predisposing or a protective polymorphism in the FRZB gene.

10   The at least one polymorphism can comprise essentially any suitable polymorphism(s), including, but not limited to, restriction fragment length polymorphisms, random amplified polymorphic DNA, arbitrary fragment length polymorphisms, simple sequence repeats, single-stranded conformation polymorphisms, and amplified variable sequences. In a preferred class of embodiments, the at least one polymorphism
15   comprises at least one single nucleotide polymorphism (SNP). For example, the polymorphism can be either allele of T2303723C, C18679T, G19524A, T19575G, T22242A, G23043A, G23415A, T23549C, A24791G, C26794G, or G27014A. In one class of embodiments, the at least one polymorphism is selected from the group consisting of: T allele of T2303723C, C allele of T2303723C, C allele of C18679T, T allele of C18679T,
20   G allele of G19524A, A allele of G19524A, T allele of T22242A, A allele of T22242A, A allele of A24791G, and G allele of A24791G.

In one class of embodiments, the presence of two or more polymorphisms is detected (e.g., two or more polymorphisms at a single polymorphic site and/or at different polymorphic sites, e.g., a haplotype). At least one of the two or more polymorphisms
25   (e.g., all of the polymorphisms) is optionally selected from the group consisting of: the T allele of T2303723C, the C allele of T2303723C, the C allele of C18679T, the T allele of C18679T, the G allele of G19524A, the A allele of G19524A, the T allele of T22242A, the A allele of T22242A, the A allele of A24791G, and the G allele of A24791G.

The nucleic acid sample typically comprises DNA or RNA. The presence of the at least
30   one polymorphism in the nucleic acid sample can be detected by any of the variety of methods known in the art. For example, the at least one polymorphism can be detected by sequencing, e.g., sequencing of the region of the FRZB gene including the polymorphic site(s). The region of FRZB is optionally amplified prior to the sequencing step. As another example, the at least one polymorphism is detected by amplification,

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e.g., of the region of the FRZB gene including the polymorphic site(s). The amplification can be, e.g., an allele-specific amplification. The amplification can comprise a polymerase chain reaction (e.g., kinetic PCR), a ligase chain reaction, or the like. As yet another example, the polymorphism can be detected by hybridization of a

5   nucleic acid probe. Thus, in one class of embodiments, to detect the polymorphism the nucleic acid sample is contacted with at least one sequence-specific oligonucleotide under conditions (e.g., stringent conditions) that allow binding of the at least one oligonucleotide to the nucleic acid sample. The at least one sequence-specific oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene

10   comprising the at least one obesity-related polymorphism. Hybridization of the at least one oligonucleotide to the nucleic acid sample is then detected. In a related class of embodiments, the FRZB region comprising the polymorphism(s) is amplified prior to probe hybridization. Thus, in this class of embodiments, the nucleic acid sample is amplified to provide an amplified nucleic acid sample. The amplified nucleic acid

15   sample is contacted with at least one sequence-specific oligonucleotide under conditions that allow binding of the oligonucleotide to the amplified nucleic acid sample (e.g., stringent conditions). The at least one sequence-specific oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the at least one obesity-related polymorphism. Hybridization of the at least one sequence-specific

20   oligonucleotide to the amplified nucleic acid sample is detected. The presence of the at least one obesity-related polymorphism can be, e.g., qualitatively or quantitatively detected.

In certain embodiments, the presence of the polymorphism inherited from one of the individual's parents provides an indication of the individual's risk for obesity (e.g., when

25   the associated FRZB allele exerts a dominant effect, such that inheritance of the polymorphism from one parent is sufficiently predictive). In other embodiments, the presence of the polymorphism inherited from both of the individual's parents provides an indication of the individual's risk for obesity.

As noted, the methods are optionally combined with known clinical methods, e.g., to

30   diagnose obesity. Thus, the methods optionally include performing at least one clinical test for obesity (e.g., determining a body mass index (BMI) of the individual).

A second general class of embodiments provides methods for determining an individual's risk for osteoporosis. In the methods, presence of at least one osteoporosis-related polymorphism in a frizzled-related protein (FRZB) gene in a nucleic acid sample

of the individual is detected. The presence of the at least one polymorphism provides an indication of the individual's risk for osteoporosis. The individual's risk for osteoporosis can be, e.g., either an increased risk or a decreased risk as compared to an individual without the at least one polymorphism (e.g., an individual with a different

5   allele at that polymorphic site). Accordingly, the at least one polymorphism can comprise a predisposing or a protective polymorphism in the FRZB gene.

The at least one polymorphism can comprise essentially any suitable polymorphism(s), including, but not limited to, restriction fragment length polymorphisms, random amplified polymorphic DNA, arbitrary fragment length polymorphisms, simple

10   sequence repeats, single-stranded conformation polymorphisms, and amplified variable sequences. In a preferred class of embodiments, the at least one polymorphism comprises at least one single nucleotide polymorphism (SNP). For example, the polymorphism can be either allele of T2303723C, C18679T, G19524A, T19575G, T22242A, G23043A, G23415A, T23549C, A24791G, C26794G, or G27014A. In one class

15   of embodiments, the at least one polymorphism is selected from the group consisting of: C allele of C18679T, T allele of C18679T, G allele of G19524A, A allele of G19524A, A allele of A24791G, G allele of A24791G, C allele of C26794G, G allele of C26794G, G allele of G27014A, and A allele of G27014A.

In one class of embodiments, the presence of two or more polymorphisms is detected

20   (e.g., two or more polymorphisms at a single polymorphic site and/or at different polymorphic sites, e.g., a haplotype). At least one of the two or more polymorphisms (e.g., all of the polymorphisms) is optionally selected from the group consisting of: the C allele of C18679T, the T allele of C18679T, the G allele of G19524A, the A allele of G19524A, the A allele of A24791G, the G allele of A24791G, the C allele of C26794G, the

25   G allele of C26794G, the G allele of G27014A, and the A allele of G27014A.

The nucleic acid sample typically comprises DNA or RNA. The presence of the at least one polymorphism in the nucleic acid sample can be detected by any of the variety of methods known in the art. For example, the at least one polymorphism can be detected by sequencing, e.g., sequencing of the region of the FRZB gene including the

30   polymorphic site(s). The region of FRZB is optionally amplified prior to the sequencing step. As another example, the at least one polymorphism is detected by amplification, e.g., of the region of the FRZB gene including the polymorphic site(s). The amplification can be, e.g., an allele-specific amplification. The amplification can comprise a polymerase chain reaction (e.g., kinetic PCR), a ligase chain reaction, or the

like. As yet another example, the polymorphism can be detected by hybridization of a nucleic acid probe. Thus, in one class of embodiments, to detect the polymorphism the nucleic acid sample is contacted with at least one sequence-specific oligonucleotide under conditions (e.g., stringent conditions) that allow binding of the at least one

5   oligonucleotide to the nucleic acid sample. The at least one sequence-specific oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the at least one osteoporosis-related polymorphism. Hybridization of the at least one oligonucleotide to the nucleic acid sample is then detected. In a related class of embodiments, the FRZB region comprising the polymorphism(s) is amplified prior to

10 probe hybridization. Thus, in this class of embodiments, the nucleic acid sample is amplified to provide an amplified nucleic acid sample. The amplified nucleic acid sample is contacted with at least one sequence-specific oligonucleotide under conditions that allow binding of the oligonucleotide to the amplified nucleic acid sample (e.g., stringent conditions). The at least one sequence-specific oligonucleotide hybridizes

15 under stringent conditions to a region of the FRZB gene comprising the at least one osteoporosis-related polymorphism. Hybridization of the at least one sequence-specific oligonucleotide to the amplified nucleic acid sample is detected. The presence of the at least one osteoporosis-related polymorphism can be, e.g., qualitatively or quantitatively detected.

20 In certain embodiments, the presence of the polymorphism inherited from one of the individual's parents provides an indication of the individual's risk for osteoporosis (e.g., when the associated FRZB allele exerts a dominant effect, such that inheritance of the polymorphism from one parent is sufficiently predictive). In other embodiments, the presence of the polymorphism inherited from both of the individual's parents provides

25 an indication of the individual's risk for osteoporosis.

As noted, the methods are optionally combined with known clinical methods, e.g., to diagnose osteoporosis. Thus, the methods optionally include performing at least one clinical test for osteoporosis (e.g., a bone-turnover assay or a bone scan).

Yet another general class of embodiments provides methods for determining an

30 individual's risk for obesity and/or osteoporosis. In the methods, the individual's genotype at one or more polymorphic sites in an FRZB gene is determined. A first genotype at the one or more polymorphic sites is statistically associated with an increased risk for obesity and/or osteoporosis as compared to a second genotype at the one or more polymorphic sites. Thus, for example, if the individual's genotype

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corresponds to the first genotype, the individual's risk for obesity and/or osteoporosis is greater than that of other individuals who have the second genotype.

Each polymorphic site can comprise one or more nucleotides. In a preferred class of embodiments, at least one of the one or more polymorphic sites consists of a single
5 nucleotide position (i.e., the individual is genotyped for one or more SNPs, e.g., a plurality and/or a haplotype of SNPs). In certain embodiments, a plurality of the polymorphic sites each consists of a single nucleotide position (e.g., position 2628, 18679, 19524, 19575, 22242, 23043, 23415, 23549, 24791, 26794, or 27014 of SEQ ID NO:1). For example, at least one of the one or more polymorphic sites can be selected
10 from the group consisting of: nucleotide position 2628, nucleotide position 18679, nucleotide position 19524, nucleotide position 22242, nucleotide position 24791, nucleotide position 26794, and nucleotide position 27014 of SEQ ID NO:1.

In some embodiments, the presence of a single allele of a particular polymorphism is sufficient to indicate whether the individual's risk of obesity and/or osteoporosis is
15 increased or decreased. In other embodiments, two copies of an allele of a particular polymorphism must be present to indicate an increased or decreased risk of obesity and/or polymorphism (e.g., when the effect is recessive such that both homologous chromosomes must carry the allele). Thus, in one class of embodiments, the first genotype is statistically associated with an increased risk for obesity as compared to the
20 second genotype. In one class of example embodiments, the first genotype comprises two T alleles and the second genotype two C alleles or one T allele and one C allele of T2303723C; the first genotype comprises two T alleles and the second genotype two C alleles or one T allele and one C allele of SNP C18679T; the first genotype comprises two A alleles and the second genotype two G alleles or one A allele and one G allele of SNP
25 G19524A; the first genotype comprises two A alleles and the second genotype two T alleles or one A allele and one T allele of SNP T22242A; and/or the first genotype comprises two G alleles and the second genotype two A alleles or one G allele and one A allele of SNP A24791G. In a related class of embodiments, the first genotype is statistically associated with an increased risk for osteoporosis as compared to the second
30 genotype. The first genotype comprises two C alleles and the second genotype two T alleles or one T allele and one C allele of SNP C18679T; the first genotype comprises two G alleles and the second genotype two A alleles or one A allele and one G allele of SNP G19524A; the first genotype comprises two A alleles and the second genotype two G alleles or one G allele and one A allele of SNP A24791G; the first genotype comprises
35 two C alleles and the second genotype two G alleles or one C allele and one G allele of

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SNP C26794G; and/or the first genotype comprises two G alleles and the second
genotype two A alleles or one G allele and one A allele of SNP G27014A.

Determining the individual's genotype typically involves obtaining a nucleic acid sample
from the individual. Determining the individual's genotype can involve amplifying at

5   least a portion of the FRZB gene from the nucleic acid sample, the portion comprising at
least one of the one or more polymorphic sites. Such amplification can be, e.g., to
directly determine the genotype or to facilitate detection of one or more polymorphisms
by an additional step. In one class of embodiments, the individual's genotype is
determined by performing an allele-specific amplification or an allele-specific extension

10   reaction. In another class of embodiments, the individual's genotype is determined by
sequencing at least a portion of the FRZB gene from the nucleic acid sample, the portion
comprising at least one of the one or more polymorphic sites. In yet another class of
embodiments, the individual's genotype is determined by hybridization of a nucleic acid
probe, optionally after amplification of at least a portion of the FRZB gene. In one class

15   of example embodiments, at least one of the one or more polymorphic sites consists of a
single nucleotide position. In these embodiments, the nucleic acid sample is contacted
with at least one sequence-specific oligonucleotide under stringent conditions. The
oligonucleotide hybridizes under the stringent conditions to the nucleic acid sample
when a first nucleotide occupies the nucleotide position defining the polymorphic site

20   but not when a second nucleotide occupies the nucleotide position. Hybridization of
the oligonucleotide to the nucleic acid sample is detected.

One aspect of the invention provides kits for detecting presence of a first predisposing or
protective polymorphism in an FRZB gene, e.g., in a nucleic acid sample of an individual
whose risk for osteoporosis and/or obesity is being assessed. Thus, one general class of

25   embodiments provides a kit including one or more first oligonucleotides capable of
detecting the first polymorphism and instructions for detecting the first polymorphism
with the one or more first oligonucleotides and for correlating said detection to the
individual's risk for osteoporosis and/or obesity, packaged in one or more containers.

Essentially all of the features noted for the method embodiments above apply to this

30   embodiment as well, as relevant. For example, in a preferred class of embodiments, the
first polymorphism is a single nucleotide polymorphism, e.g., a SNP selected from the
group consisting of: the T allele of T2303723C, the C allele of T2303723C, the C allele of
C18679T, the T allele of C18679T, the G allele of G19524A, the A allele of G19524A, the
T allele of T22242A, the A allele of T22242A, the A allele of A24791G, the G allele of

A24791G, the C allele of C26794G, the G allele of C26794G, the G allele of G27014A, and the A allele of G27014A. Other potential SNPs include, but are not limited to, either allele of T19575G, G23043A, G23415A, and T23549C.

5   In one aspect, the kit can be used to detect the presence of the first polymorphism by hybridization of a nucleic acid probe to the polymorphism. Thus, in one class of embodiments, the one or more first oligonucleotides comprise at least one probe. In certain embodiments, the first oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the first polymorphism. In one class of embodiments, the first polymorphism is a first single nucleotide polymorphism

10   comprising a first nucleotide at a first nucleotide position. In this class of embodiments, under stringent conditions, the first oligonucleotide hybridizes to a region of the FRZB gene comprising the first single nucleotide polymorphism with a signal to noise ratio that is at least 2x (e.g., at least 5x or at least 10x) the signal to noise ratio at which the first oligonucleotide hybridizes to the region of the FRZB gene comprising a second

15   nucleotide at the first nucleotide position.. The first oligonucleotide is typically fully complementary to the region of the FRZB gene comprising the first polymorphism, and typically comprises at least about 10 contiguous nucleotides complementary to the FRZB gene.

To facilitate detection of the polymorphism (e.g., through detection of hybridization

20   between the one or more first oligonucleotides and a nucleic acid comprising the polymorphism), for example, the one or more first oligonucleotides optionally comprise a label, e.g., an isotopic, fluorescent, fluorogenic, luminescent or colorimetric label. In some embodiments, the label itself directly produces a detectable signal (e.g., a fluorescent label). In other embodiments, the kit also includes a reagent that detects the

25   label (e.g., an enzyme that cleaves a colorimetric label, a binding moiety, or the like).

In one aspect, the one or more first oligonucleotides comprise one or more primers. The primer(s) can be used to detect the polymorphism, e.g., in an allele-specific amplification or extension reaction. For example, in one class of embodiments, the first polymorphism is a first single nucleotide polymorphism comprising a first nucleotide at

30   a first nucleotide position, and the 3' nucleotide of one of the one or more first oligonucleotides is complementary to the first nucleotide.

The primer(s) can be used to amplify a region of FRZB comprising the polymorphism, e.g., for subsequent detection of the polymorphism by hybridization, sequencing, or the

like. In one class of embodiments, the one or more first oligonucleotides comprise amplification primers, wherein the amplification primers amplify a nucleic acid sequence comprising the first polymorphism. In a related class of embodiments, the one or more first oligonucleotides comprise sequencing primers that flank the first

5 polymorphism.

The one or more first oligonucleotides are optionally immobilized on a substrate. The substrate can be, for example, a planar substrate or a beaded substrate. The oligonucleotide(s) can be arranged in an array of other oligonucleotides used to detect other polymorphisms, e.g., other polymorphisms in FRZB.

10 The kit can optionally be used to detect more than one polymorphism (simultaneously or sequentially). Thus, in one class of embodiments, the kit also includes one or more second oligonucleotides capable of detecting a second polymorphism (and optionally third, fourth, fifth, etc. oligonucleotides capable of detecting third, fourth, fifth, etc. polymorphisms). The second polymorphism can be at the same polymorphic site as the

15 first or at a different polymorphic site (in FRZB or a different gene), and can be protective or predisposing.

One general class of embodiments provides arrays for detecting presence of one or more predisposing and/or protective polymorphisms in an FRZB gene, e.g., in a nucleic acid sample of an individual whose risk for osteoporosis and/or obesity is being assessed. In

20 one class of embodiments, the array comprises a substrate and a plurality of oligonucleotides, each of which oligonucleotides hybridizes to a region of the FRZB gene comprising at least one of the polymorphisms. The hybridization detects the presence of the polymorphism, and this detection provides an indication of the individual's risk for osteoporosis and/or obesity. The plurality of oligonucleotides are immobilized on the

25 substrate. Typically, the array is used for detecting the presence of a plurality of polymorphisms, e.g., multiple alleles at a single polymorphic site and/or different polymorphic sites.

Essentially all of the features noted for the method and kit embodiments above apply to this embodiment as well, as relevant. For example, the one or more polymorphisms

30 preferably comprise one or more single nucleotide polymorphisms. For example, at least one of the one or more polymorphisms can be selected from the group consisting of: the T allele of T2303723C, the C allele of T2303723C, the C allele of C18679T, the T allele of C18679T, the G allele of G19524A, the A allele of G19524A, the T allele of

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T22242A, the A allele of T22242A, the A allele of A24791G, the G allele of A24791G, the C allele of C26794G, the G allele of C26794G, the G allele of G27014A, and the A allele of G27014A. Other potential SNPs include, but are not limited to, either allele of T19575G, G23043A, G23415A, and T23549C.

5   In one class of embodiments in which the array can be used to detect presence of one or more SNPs, each of the oligonucleotides in the array hybridizes under stringent conditions to a region of the FRZB gene comprising one of the single nucleotide polymorphisms with a signal to noise ratio that is at least 2x (e.g., at least 5x or at least 10x) that at which the oligonucleotide hybridizes to a region of the FRZB gene

10   comprising any of the remaining single nucleotide polymorphisms. Typically, one oligonucleotide is used to detect one SNP; that is, each of the oligonucleotides typically hybridizes to a distinct single nucleotide polymorphism.

As noted, the plurality of oligonucleotides are immobilized on a substrate, e.g., a planar substrate, a membrane, a glass slide, or the like. Typically, each of the plurality of

15   oligonucleotides is immobilized at a known, pre-determined position on the substrate.

To facilitate detection of polymorphisms by specific hybridization with the oligonucleotides, each of the plurality of oligonucleotides is typically fully complementary to a region of the FRZB gene comprising one of the polymorphisms, and each of the plurality of oligonucleotides typically comprises at least about 10

20   contiguous nucleotides complementary to the FRZB gene. Each of the plurality of oligonucleotides optionally comprises a label, e.g., a label that facilitates detection of hybridization between the oligonucleotide and the corresponding polymorphism.

The array is optionally part of a system. Thus, one class of embodiments provides a system comprising an array of the invention and system instructions that correlate the

25   detection of the presence of one or more predisposing or protective polymorphisms to the individual's risk for osteoporosis and/or obesity.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts exemplary FRZB SNPs. Nine base pairs centered on each SNP are

30   shown; the upper strand corresponds to the strand whose sequence is listed in SEQ ID NO:1. SNP alleles illustrated are: C allele of C18679T (Panel A), T allele of C18679T (Panel B), G allele of G19524A (Panel C), A allele of G19524A (Panel D), T allele of

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T19575G (Panel E), G allele of T19575G (Panel F), T allele of T22242A (Panel G), A allele of T22242A (Panel H), G allele of G23043A (Panel I), A allele of G23043A (Panel J), G allele of G23415A (Panel K), A allele of G23415A (Panel L), T allele of T23549C (Panel M), C allele of T23549C (Panel N), A allele of A24791G (Panel O), G allele of A24791G (Panel P), C allele of C26794G (Panel Q), G allele of C26794G (Panel R), G allele of G27014A (Panel S), A allele of G27014A (Panel T), T allele of T2303723C (Panel U), and C allele of T2303723C (Panel V).

DEFINITIONS

Before describing the invention in detail, it is to be understood that this invention is not limited to particular devices or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. The following definitions supplement those in the art and are directed to the current application and are not to be imputed to any related or unrelated case, e.g., to any commonly owned patent or application. Although any methods and materials similar or equivalent to those described herein can be used in the practice for testing of the invention, the preferred materials and methods are described herein. In describing and claiming the invention, the following terminology will be used in accordance with the definitions set out below.

As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "a cell" includes a combination of two or more cells; reference to "bacteria" includes mixtures of bacteria, and the like.

As used herein, the terms "nucleic acid," "polynucleotide" and "oligonucleotide" refer to single-stranded or double-stranded nucleotide polymers comprised of more than two nucleotide subunits covalently joined together. The nucleotides may comprise deoxyribonucleotides (containing 2-deoxy-D-ribose), ribonucleotides (containing D-ribose), and/or any other N-glycoside of a purine or pyrimidine base, or modified purine or pyrimidine bases, or any combination thereof. The sugar groups of the nucleotide subunits may also comprise modified derivatives of ribose or deoxyribose,

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such as O-methyl ribose. The nucleotide subunits of an oligonucleotide may be joined
by phosphodiester linkages, phosphorothioate linkages, methyl phosphonate linkages or
by other linkages, including, but not limited to, rare or non-naturally-occurring
linkages, that do not prevent hybridization of the oligonucleotide. Furthermore, an

5   oligonucleotide may have uncommon nucleotides or non-nucleotide moieties. With the
addition of such analogs as locked nucleic acids (LNAs), the size of primers and probes
can be reduced to as little as 8 nucleic acids. Locked nucleic acids are a novel class of
bicyclic DNA analogs in which the 2' and 4' positions in the furanose ring are joined via
an O-methylene (oxy-LNA), S-methylene (thio-LNA), or amino methylene (amino-

10   LNA) moiety.

Oligonucleotide probes and amplification oligonucleotides of a defined sequence may be
produced by techniques known to those of ordinary skill in the art, such as by chemical
or biochemical synthesis, and by in vitro or in vivo expression from recombinant nucleic
acid molecules, e.g., bacterial or retroviral vectors. As used herein, an oligonucleotide

15   does not consist of wild-type chromosomal DNA or the in vivo transcription products
thereof.

Oligonucleotides which are primer and/or probe sequences, as described below, may
comprise DNA, RNA or nucleic acid analogs such as uncharged nucleic acid analogs
including, but not limited to, peptide nucleic acids (PNAs), which are disclosed in

20   International Patent Application WO 92/20702, or morpholino analogs, which are
described in U.S. Pat. Nos. 5,185,444, 5,034,506, and 5,142,047, all of which are herein
incorporated by reference in their entireties. Such sequences can routinely be
synthesized using a variety of techniques currently available. For example, a sequence of
DNA can be synthesized using conventional nucleotide phosphoramidite chemistry and

25   the instruments available from Applied Biosystems, Inc. (Foster City, Calif.); DuPont
(Wilmington, Del.); or Milligen (Bedford, Mass.). Similarly, and when desirable, the
sequences can be labeled using methodologies well known in the art such as described in
U.S. Pat. Nos. 5,464,746; 5,424,414; and 4,948,882, all of which are herein incorporated
by reference in their entireties. Oligonucleotides (including, e.g., labeled or modified

30   oligos) can also be ordered from a variety of commercial sources known to persons of
skill. Essentially any nucleic acid can be custom ordered from any of a variety of
commercial sources, for example, The Midland Certified Reagent Company
(www.mcrc.com), The Great American Gene Company (www.genco.com), ExpressGen
Inc. (www.expressgen.com), and QIAGEN (http://oligos.qiagen.com), among many

35   others.

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A nucleic acid, nucleotide, polynucleotide or oligonucleotide can comprise the five biologically occurring bases (adenine, guanine, thymine, cytosine and uracil) and/or bases other than the five biologically occurring bases. These bases may serve a number of purposes, e.g., to stabilize or destabilize hybridization; to promote or inhibit probe

5   degradation; or as attachment points for detectable moieties or quencher moieties. For example, a polynucleotide of the invention can contain one or more modified, non-standard, or derivatized base moieties, including, but not limited to, N^6-methyl-adenine, N^6-tert-butyl-benzyl-adenine, imidazole, substituted imidazoles, 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine,

10   5-(carboxyhydroxymethyl)uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-methyladenine, 7-methylguanine, 5-methylaminomethyluracil, 5-

15   methoxyaminomethyl-2-thiouracil, beta-D mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6- isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5- oxyacetic acidmethylester, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, 2,6-

20   diaminopurine, and 5-propynyl pyrimidine. Other examples of modified, non-standard, or derivatized base moieties may be found in U.S. Patent Nos. 6,001,611, 5,955,589, 5,844,106, 5,789,562, 5,750,343, 5,728,525, and 5,679,785, each of which is incorporated herein by reference in its entirety.

Furthermore, a nucleic acid, nucleotide, polynucleotide or oligonucleotide can comprise

25   one or more modified sugar moieties including, but not limited to, arabinose, 2-fluoroarabinose, xylulose, and hexose. A nucleic acid, nucleotide, polynucleotide or oligonucleotide can comprise phosphodiester linkages or modified linkages including, but not limited to phosphotriester, phosphoramidate, siloxane, carbonate, carboxymethylester, acetamidate, carbamate, thioether, bridged phosphoramidate,

30   bridged methylene phosphonate, phosphorothioate, methylphosphonate, phosphorodithioate, bridged phosphorothioate or sulfone linkages, and combinations of such linkages.

An "isolated" nucleic acid molecule, as used herein, is one that is separated from nucleotide sequences which normally flank the nucleic acid molecule and/or has been

completely or partially purified from other biological material (*e.g.*, protein) normally
associated with the nucleic acid.

The nucleic acid molecule can be fused to other coding or regulatory sequences and still
be considered isolated. Thus, recombinant DNA contained in a vector is included in the

5   definition of "isolated" as used herein. Also, isolated nucleic acid molecules include
recombinant DNA molecules in heterologous host cells, as well as partially or
substantially purified DNA molecules in solution. "Isolated" nucleic acid molecules also
encompass *in vivo* and *in vitro* RNA transcripts of the DNA molecules of the invention.
An isolated nucleic acid molecule or nucleotide sequence can include a nucleic acid

10   molecule or nucleotide sequence that is synthesized chemically or by recombinant
means. Also, isolated polynucleotides include recombinant DNA molecules in
heterologous organisms, as well as partially or substantially purified DNA molecules in
solution. *In vivo* and *in vitro* RNA transcripts of the DNA molecules of the invention are
also encompassed by "isolated" nucleotide sequences. Such polynucleotides are useful,

15   *e.g.*, as primers and/or probes for detecting polymorphisms, in the manufacture of the
encoded polypeptide, as probes for isolating homologous sequences (*e.g.*, from other
mammalian species), for gene mapping (*e.g.*, by *in situ* hybridization with
chromosomes), or for detecting expression of the gene in tissue (*e.g.*, human tissue),
such as by Northern blot analysis.

20   The isolated nucleic acid molecules may be RNA, mRNA, DNA, or cDNA, for example,
and may be double- or single-stranded. They may encode the sense strand, the non-
coding regions, and/or the antisense strand. The nucleic acid molecule can include all or
a portion of the coding sequence of the gene and can further comprise additional non-
coding regions such as introns and non-coding 3' and 5' sequences (including regulatory

25   sequences, for example). Additionally, the nucleic acid molecule can be fused to a
marker sequence, for example, a sequence that can be used to purify the nucleic acid
molecule.

The nucleic acid molecules of the invention can comprise one or more modified
nucleotide residues. The modification may be at the base, sugar and/or phosphate

30   moiety and include, for example, halogenation, hydroxylation, alkylation, an attached
linker and/or label. The modifications can further comprise, for example, labeling,
methylation, internucleotide modifications such as uncharged linkages (*e.g.*, methyl
phosphonates, phosphotriesters, phosphoamidates, carbamates), charged linkages (*e.g.*,
phosphorothioates, phosphorodithioates), pendent moieties (*e.g.*, polypeptides),

intercalators (*e.g.*, acridine, psoralen), chelators, alkylators, and modified linkages (*e.g.*, alpha anomeric nucleic acids). Also included are synthetic molecules that mimic nucleic acid molecules in the ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules include, for example, those in which

5   peptide linkages substitute for phosphate linkages in the backbone of the molecule.

In certain embodiments, nucleic acid molecules of the invention include, but are not limited to, FRZB mRNA, cDNA and/or genomic DNA molecules. Nucleic acid molecules of the invention also include oligonucleotides, e.g., an oligonucleotide comprising one or more of the FRZB SNPs described herein.

10   As used herein, the term "primer" refers to an oligonucleotide having a hybridization specificity sufficient for the initiation of an enzymatic polymerization under predetermined conditions, for example in an amplification technique such as polymerase chain reaction (PCR), in a process of sequencing, in a method of reverse transcription and/or the like.

15   As used herein, the term "probe" refers to an oligonucleotide having a hybridization specificity sufficient for binding to a defined target sequence under predetermined conditions, for example in an amplification technique such as a 5'-nuclease reaction, in a hybridization-dependent detection method, such as a Southern or Northern blot, and/or the like. Primers and probes may be used in a variety of ways and may be defined by the

20   specific use. For example, a "capture probe" is immobilized or can be immobilized on a solid support by any appropriate means, including, but not limited to: by covalent bonding, by adsorption, by hydrophobic and/or electrostatic interaction, or by direct synthesis on a solid support (see in particular patent application WO 92 10092). A "detection probe" may be labeled by means of a marker chosen, for example, from

25   radioactive isotopes, enzymes, in particular enzymes capable of acting on a chromogenic, fluorescent or luminescent substrate (in particular a peroxidase or an alkaline phosphatase), chromophoric chemical compounds, chromogenic, fluorigenic or luminescent compounds, analogues of nucleotide bases, and ligands such as biotin. Illustrative fluorescent compounds include, for example, fluorescein,

30   carboxyfluorescein, tetrachlorofluorescein, hexachlorofluorescein, Cy3, tetramethylrhodamine, Cy3.5, carboxy-x-rhodamine, Texas Red, Cy5, and Cy5.5. Illustrative luminescent compounds include, for example, luciferin and 2,3-dihydrophthalazinediones, such as luminol.

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All of the oligonucleotides, primers and probes of the invention, whether hybridization assay probes, amplification primers, or other oligonucleotides, may be modified with chemical groups to enhance their performance or to facilitate the characterization of amplification products. For example, backbone-modified oligonucleotides such as those

5   having phosphorothioate or methylphosphonate groups which render the oligonucleotides resistant to the nucleolytic activity of certain polymerases or to nuclease enzymes may allow the use of such enzymes in an amplification or other reaction. Another example of modification involves using non-nucleotide linkers (*e.g.*, Arnold, *et al.*, "Non- Nucleotide Linking Reagents for Nucleotide Probes", EP 0 313 219 hereby

10   incorporated by reference herein in its entirety) incorporated between nucleotides in the nucleic acid chain which do not interfere with hybridization or the elongation of the primer. Amplification oligonucleotides may also contain mixtures of the desired modified and natural nucleotides.

The 3' end of an amplification primer or a probe may optionally be blocked to prevent
15   initiation of DNA synthesis as described by McDonough, *et al.*, entitled "Nucleic Acid Sequence Amplification", WO94/03472 which enjoys common ownership with the invention and is hereby incorporated by reference herein in its entirety. A mixture of different 3' blocked amplification oligonucleotides, or of 3' blocked and unblocked oligonucleotides, may increase the efficiency of nucleic acid amplification, as described
20   therein. The 5' end of the oligonucleotides may be modified to be resistant to the 5'-exonuclease activity present in some nucleic acid polymerases. Such modifications can be carried out by adding a non-nucleotide group to the terminal 5' nucleotide of the primer using techniques such as those described by Arnold, et al., *supra*, entitled "Non-Nucleotide Linking Reagents for Nucleotide Probes", incorporated by reference herein.

25   Once synthesized, selected oligonucleotide probes may be labeled by any of several well-known methods (*e.g.*, J. Sambrook, *infra*). Useful labels include radioisotopes as well as non-radioactive reporting groups. Isotopic labels include ^{3}H, ^{35}S, ^{32}P, ^{125}I, ^{57}Co and ^{14}C. Isotopic labels can be introduced into the oligonucleotide by techniques known in the art such as nick translation, end labeling, second strand synthesis, the use of reverse
30   transcription, and by chemical methods. When using radiolabeled probes hybridization can be detected, e.g., by autoradiography, scintillation counting, or gamma counting. The detection method selected will depend upon the particular radioisotope used for labeling.

Non-isotopic materials can also be used for labeling and may be introduced internally into the nucleic acid sequence or at the end of the nucleic acid sequence. Modified nucleotides may be incorporated enzymatically or chemically. Chemical modifications of the probe may be performed during or after synthesis of the probe, for example,

5   through the use of non-nucleotide linker groups as described by Arnold, *et al.*, *supra* "Non-Nucleotide Linking Reagents for Nucleotide Probes," incorporated by reference herein. Non-isotopic labels include fluorescent molecules; chemiluminescent molecules, enzymes, cofactors, enzyme substrates, haptens or other ligands.

In one embodiment, the probes are labeled with an acridinium ester. Acridinium ester

10   labeling may be performed as described by Arnold et al., U.S. Pat. No. 5,185,439, entitled "Acridinium Ester Labeling and Purification of Nucleotide Probes," issued Feb. 9, 1993 and hereby incorporated by reference herein in its entirety.

The term "sequence-specific oligonucleotide" refers to an oligonucleotide that hybridizes (under defined conditions) to a target nucleic acid with a signal to noise ratio

15   at least 2x higher than a signal to noise ratio at which it hybridizes to a nucleic acid that is not the target. For example, a sequence-specific oligonucleotide can hybridize, under stringent conditions, to a region of FRZB comprising a first allele of a SNP (the target nucleic acid) with a signal to noise ratio at least 2x (e.g., at least 5x or 10x) higher than a signal to noise ratio at which the oligonucleotide hybridizes to the region of FRZB

20   comprising a second allele of the SNP.

The term "FRZB gene" or "FRZB locus" refers to the genomic nucleic acid sequence that encodes the FRZB protein. The nucleotide sequence of a gene, as used herein, encompasses coding regions, referred to as exons, intervening, non-coding regions, referred to as introns, and upstream and/or downstream regions. Upstream or

25   downstream regions can include regions of the gene that are transcribed but not part of an intron or exon, or regions of the gene that comprise, for example, binding sites for factors that modulate gene transcription. The sequence for the human FRZB genomic sequence is provided at GenBank accession numbers NT_005100.3, NT_005265, and NT_005403, and a portion of the sequence is provided herein as SEQ ID NO:1. The

30   sequence for the human FRZB mRNA is provided at GenBank accession number NM_001463, and is provided herein as SEQ ID NO:2.

The term "allele", as used herein, refers to a sequence variant of a gene and/or a polymorphism. Alleles are typically identified with respect to one or more polymorphic

positions, with the rest of the gene sequence unspecified. For example, a FRZB allele may be defined by the nucleotide present at a single SNP, or by the nucleotides present at a plurality of SNPs. Examples of such FRZB SNPs are provided in Table 1, below.

5   For convenience, the allele present at the higher or highest frequency in the population will be referred to as the wild-type allele, and less frequent allele(s) will be referred to as mutant allele(s). (However, it is worth noting that an allele which is more frequent in one population may be less frequent in a different population.) This designation of an allele as a mutant is meant solely to distinguish the allele from the wild-type allele and is not meant to indicate a change or loss of function.

10   The terms "polymorphic" and "polymorphism", as used herein, refer to the condition in which two or more variants of a specific genomic sequence, or the encoded amino acid sequence, can be found in a population. The terms refer either to the nucleic acid sequence or the encoded amino acid sequence; the use will be clear from the context. The term "polymorphic region" or "polymorphic site" refers to a region of the nucleic
15   acid where the nucleotide difference that distinguishes the variants occurs, or, for amino acid sequences, a region of the amino acid sequence where the amino acid difference that distinguishes the protein variants occurs. As used herein, the term "single nucleotide polymorphism", or SNP, refers to polymorphism at a polymorphic site consisting of a single nucleotide position. As will be clear from the context, the term
20   polymorphism can refer to a specific variant sequence at a polymorphic site; for example, the term SNP can refer to the specific nucleotide (e.g., A, C, G, or T) occupying a polymorphic site consisting of a single nucleotide position.

Individual amino acids in a sequence are represented herein as AN or NA, wherein A is the amino acid in the sequence and N is the position in the sequence. In the case that
25   position N is polymorphic, it is convenient to designate one variant (e.g., the more frequent variant) as A_1N and the other variant (e.g., the less frequent variant) as NA_2. Alternatively, the polymorphic site, N, is represented as A_1NA_2, wherein A_1 is the amino acid in one variant and A_2 is the amino acid in the other variant. It is worth noting that an allele which is more frequent in one population may be less frequent in a different
30   population. Either the one-letter or three-letter codes are used for designating amino acids (see Lehninger, *BioChemistry 2nd ed.*, 1975, Worth Publishers, Inc. New York, NY: pages 73-75, incorporated herein by reference). The amino acid positions are numbered based on the sequence of the mature FRZB protein.

Representations of nucleotides and single nucleotide variations in DNA sequences are analogous to the representations of amino acids. For example, C18679T represents a single nucleotide polymorphism at nucleotide position 18679, wherein cytosine is present in the more frequent (wild-type) allele in the population and thymidine is present in the less frequent (mutant) allele. In general, a SNP can be represented as A_1NA_2, wherein A_1 is the nucleotide present in one variant and A_2 is the nucleotide in the other variant. The single letter codes for the nucleotides are well known to those in the art, i.e., C for cytosine; A for adenine; T for thymidine, G for guanine, I for inosine, and U for uracil. It will be clear that in a double stranded form, the complementary strand of each allele will contain the complementary base at the polymorphic position; a SNP (or other polymorphism) can thus be described and/or detected with reference to the nucleotide(s) occupying the polymorphic site on either strand.

As used herein, the term "predisposing polymorphism" refers to a polymorphism that is positively associated with a condition, such as, for example, obesity and/or osteoporosis. The presence of a predisposing polymorphism in an individual could be indicative that the individual has an increased risk for the disease relative to an individual without the polymorphism. The term "protective polymorphism" refers to a polymorphism that is negatively associated with a condition. The presence of a protective polymorphism in an individual could be indicative that the individual has a decreased risk for the disease relative to an individual without the polymorphism.

The term "obesity-associated polymorphism" or "obesity-related polymorphism" refers to a polymorphism that is associated with obesity (e.g., with a high body mass index), either positively or negatively.

The term "osteoporosis-associated polymorphism" or "osteoporosis-related polymorphism" refers to a polymorphism that is associated with osteoporosis (e.g., with increased incidence of hip and/or vertebral fracture and/or decreased bone mineral density), either positively or negatively.

The term "association" or "associated with" in the context of this invention refers to the presence of a disease or phenotypic trait in individuals with one or more specific alleles or polymorphisms in one or more specific genes.

As used herein, the term "odds ratio" (OR) refers to the ratio of the frequency of the disease in individuals having a particular marker (allele or polymorphism) to the frequency of the disease in individuals without the marker (allele or polymorphism).

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As used herein, the term "linkage disequilibrium" (LD) refers to alleles at different loci that are not associated at random, i.e., not associated in proportion to their frequencies. If the alleles are in positive linkage disequilibrium, then the alleles occur together more often than expected, assuming statistical independence. Conversely, if the alleles are in

5   negative linkage disequilibrium, then the alleles occur together less often than expected assuming statistical independence.

The term "genotype", as used herein, refers to a description of the allele(s) of a gene or genes contained in an individual or a sample. As used herein, no distinction is made between the genotype of an individual and the genotype of a sample originating from

10   the individual. Although, typically, a genotype is determined from samples of diploid cells, a genotype can be determined from a sample of haploid cells, such as a sperm cell. Similarly, an individual's "genotype at one or more polymorphic sites" refers to a description of the allele(s) of one or more polymorphisms contained in the individual or a sample. For example, an individual's genotype for a SNP is defined by the nucleotide

15   present at that polymorphic site.

The term "haplotype", as used herein, refers to a description of the variants of a gene or genes contained on a single chromosome, i.e., the genotype of a single chromosome. A haplotype is a set of maternally inherited alleles, or a set of paternally inherited alleles, at any locus. A haplotype may also refer to two or more SNPs grouped together.

20   As used herein, the term "target region" refers to a region of a nucleic acid which is to be analyzed and usually includes at least one polymorphic region.

The term "stringent" as used herein refers to hybridization and wash conditions that are at or near the Tm for a particular sequence, taking into account considerations such as salt concentration and oligonucleotide length and base composition, for example.

25   Generally, stringent conditions are selected to be about 5 °C to 15 °C lower than the thermal melting point (Tm) for the specific sequence at a defined ionic strength and pH. Higher stringency conditions are optionally selected, for example, equal to the Tm or even 5 °C or more greater than the Tm. The Tm is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched

30   probe. Typically, stringent conditions will be those in which the salt concentration is at least about 0.02 molar at pH 7 and the temperature is at least about 50 °C for a sequence with a Tm of about 55-65 °C. As other factors may significantly affect the stringency of hybridization, including, among others, base composition, length of the nucleic acid

strands, the presence of organic solvents, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one.

A "label" is a moiety that facilitates detection of a molecule. Common labels in the context of the present invention include fluorescent, luminescent, and/or colorimetric
5   labels. Suitable labels include radionucleotides, enzymes, substrates, cofactors, inhibitors, fluorescent moieties, chemiluminescent moieties, magnetic particles, and the like. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241. Many labels are commercially available and can be used in the context of the invention.

10 A "polynucleotide sequence", "nucleotide sequence", or "nucleic acid sequence" is a polymer of nucleotides (an oligonucleotide, a DNA, a nucleic acid, etc.) or a character string representing a nucleotide polymer, depending on context. From any specified polynucleotide sequence, either the given nucleic acid or the complementary polynucleotide sequence (e.g., the complementary nucleic acid) can be determined.

15 A variety of additional terms are defined or otherwise characterized herein.

DETAILED DESCRIPTION

Certain embodiments of the invention stem from the observation that at least one
20 polymorphism in the FRZB gene is correlated with an individual's risk for obesity and/or osteoporosis. Further embodiments provide methods for detecting an individual's increased or decreased risk for obesity and/or osteoporosis. Further embodiments provide kits, reagents and arrays useful for detecting an individual's risk for obesity and/or osteoporosis.

25 In one aspect, the invention provides a method for detecting an individual's increased or decreased risk for obesity by detecting the presence of at least one obesity-associated polymorphism in the FRZB locus in a nucleic acid sample of the individual, wherein the presence of said at least one polymorphism indicates the individual's increased or decreased risk for obesity.

In a further aspect, the invention provides a method for detecting an individual's increased or decreased risk for osteoporosis by detecting the presence of at least one osteoporosis-associated polymorphism in the FRZB locus in a nucleic acid sample of the individual, wherein the presence of said at least one polymorphism indicates the

5   individual's increased or decreased risk for osteoporosis.

In a further embodiment, the invention provides a method for detecting the presence of osteoporosis in an individual by a combination of the diagnostic test for predisposition and at least one other clinical test for osteoporosis, including but not limited to: a bone-turnover assay and any type of bone scan. Other diseases, conditions or criteria which

10   may predispose for or be used in addition to the above include, but are not limited to, hyperthyroidism, posttransplantation, maladsorption, hyperparathyroidism, alcoholism, and family history.

In one embodiment, the FRZB polymorphism is selected from the FRZB polymorphisms listed in Table 1. In a further embodiment, more than one

15   polymorphism is detected in the nucleic acid sample, at least one of which is selected from the polymorphisms listed in Table 1. In a further embodiment, at least two of the polymorphisms selected from those listed in Table 1 are detected. Examples of polymorphisms in FRZB that can be used include but are not limited to: T2303723C, C18679T, G19524A, T19575G, T22242A, G23043A, G23415A, T23549C, A24791G,

20   C26794G, and G27014A.

The individual can be any mammal, including human, and belong to any race or population. The individual may be male or female. However, it is understood that the methods, kits and compositions described herein would be ideally suited for the analysis of osteoporosis in women over age 50, and/or post-menopausal women. Further, the

25   methods, kits and compositions described herein would be ideally suited to the analysis of osteoporosis in men or women between the ages of 35 and 77. More particularly, men and women over 40 years of age would be ideally suited for analysis.

In a further embodiment, men and women between birth and the age of about 75 may be screened for a predisposition to obesity, more specifically, men and women between

30   the ages of 5 and 55. Of interest would be to identify an individual predisposed to obesity at an early age and/or prenatally, in order to prevent the weight gain before it happens. In a further embodiment, the individual is identified at certain high-risk times or situations in the lifetime. Such times include but are not limited to: adolescence,

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menopause, old age, periods of depression, illnesses which predispose to obesity and/or radical life changes. In addition, before starting on a diet regimen, it may be of interest to determine a patient's predisposition to obesity, which may influence the type of program selected, speed of weight loss, and the ability of the patient to maintain a

5   different weight level.

The nucleic acid sample can be obtained from any part of the individual's body, including, but not limited to: hair, skin, nails, tissues, such as organs or tumors, or bodily fluids, such as saliva, blood, plasma, serum, spinal fluid, lymph, synovial fluid, semen, seminal fluid, bronchio-alveolar lavage, urine, or tears, as well as samples of

10   isolated blood or tissue cells or in vitro cell culture constituents (including, but not limited to, conditioned medium obtained from the growth of cells in cell culture medium, recombinant cells and cell components). The nucleic acid sample can, but need not, be amplified by any amplification method including, but not limited to, polymerase chain reaction ("PCR").

15   The polymorphism can be any predisposing or protective polymorphism in the FRZB locus. In one embodiment of the invention, the polymorphism can be any polymorphism identified as predisposing or protective by methods taught herein. In one embodiment, the polymorphism can be a single nucleotide polymorphism (SNP) in the FRZB locus. In another embodiment, specific haplotypes in the FRZB locus as well

20   as specific combinations of, and interactions between, SNPs at this locus can be indicative of an increased or a decreased risk of obesity and/or osteoporosis.

In a further embodiment, the presence of the polymorphism from only one parent is sufficiently predictive. In a further embodiment, the presence of the polymorphism from both parents is sufficiently predictive.

25   The polymorphism can be detected by any method known in the art for detecting the presence of a specific polymorphism in a nucleic acid sample. These methods include, but are not limited to, contacting the nucleic acid sample with one or more nucleic acid molecules that hybridize under stringent hybridization conditions to at least one FRZB polymorphism and detecting the hybridization, detection by amplification of the nucleic

30   acid sample by, for example, PCR, and by direct sequencing of the nucleic acid sample.

In certain embodiments, an individual's risk for osteoporotic or obesity-related diseases is diagnosed from the individual's FRZB genotype. An individual who has at least one polymorphism statistically associated with osteoporosis and/or obesity possesses a factor

contributing to either an increased or a decreased risk as compared to an individual
without the polymorphism. The statistical association of various FRZB polymorphisms
(sequence variants) with obesity and/or osteoporosis is shown in the examples.

The genotype can be determined using any method capable of identifying nucleotide
5   variation, e.g., nucleotide variation consisting of single nucleotide polymorphic sites.
The particular method used is not a critical aspect of the invention. A number of
suitable methods are described below.

In one embodiment of the invention, genotyping is carried out using oligonucleotide
probes specific to variant FRZB sequences. In one embodiment, a region of the FRZB
10   gene which encompasses one or several polymorphic sites of interest is amplified prior
to, or concurrent with, the hybridization of probes directed to such sites. Probe-based
assays for the detection of sequence variants are well known in the art.

Alternatively, genotyping is carried out using allele-specific amplification or extension
reactions, wherein allele-specific primers are used which support primer extension only
15   if the targeted allele is present. Typically, an allele-specific primer hybridizes to the
FRZB gene such that the 3' terminal nucleotide aligns with a polymorphic position.
Allele-specific amplification reactions and allele-specific extension reactions are well
known in the art.

Another aspect of the invention relates to a kit useful for detecting the presence of a
20   predisposing or a protective polymorphism in the FRZB locus in a nucleic acid sample
of an individual whose risk for osteoporosis and/or obesity is being assessed. The kit can
comprise one or more oligonucleotides capable of detecting a predisposing or protective
polymorphism in the FRZB locus as well as instructions for using the kit to detect
susceptibility to obesity and/or osteoporosis. In preferred embodiments, the
25   oligonucleotide or oligonucleotides each individually comprise a sequence that
hybridizes under stringent hybridization conditions to at least one FRZB
polymorphism. In some embodiments, the oligonucleotide or oligonucleotides each
individually comprise a sequence that is fully complementary to a nucleic acid sequence
comprising a FRZB polymorphism.

30   In some embodiments, the oligonucleotide can be used to detect the presence of the
FRZB polymorphism by hybridizing to the polymorphism under stringent hybridizing
conditions. In some embodiments, the oligonucleotide can be used as an extension

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primer in either an amplification reaction such as PCR or a sequencing reaction, wherein FRZB polymorphism is detected either by amplification or sequencing.

In certain embodiments, the kit can further comprise amplification or sequencing primers which can, but need not, be sequence-specific. The kit can also comprise reagents for labeling one or more of the oligonucleotides, or comprise labeled oligonucleotides. Optionally, the kit can comprise reagents to detect the label.

In some embodiments, the kit can comprise one or more oligonucleotides that can be used to detect the presence of two or more predisposing or protective FRZB polymorphisms or combinations of predisposing polymorphisms, protective polymorphisms or both.

In another aspect, the invention provides an array useful for detecting the presence of a predisposing or a protective FRZB polymorphism in a nucleic acid sample of an individual whose risk for obesity and/or osteoporosis is being assessed. The array can comprise one or more oligonucleotides capable of detecting a predisposing or protective FRZB polymorphism. The oligonucleotides can be immobilized on a substrate, e.g., a membrane or glass. In preferred embodiments, the oligonucleotide or oligonucleotides each individually comprise a sequence that can hybridize under stringent hybridization conditions to a nucleic acid sequence comprising a FRZB polymorphism. In some embodiments, the oligonucleotide or oligonucleotides each individually comprise a sequence that is fully complementary to a nucleic acid sequence comprising a FRZB polymorphism. The oligonucleotide or oligonucleotides can, but need not, be labeled. In some embodiments, the array can be a microarray.

In some embodiments, the array can comprise one or more oligonucleotides used to detect the presence of two or more predisposing or protective FRZB polymorphisms or combinations of predisposing polymorphisms, protective polymorphisms or both.

One aspect of the invention provides nucleic acids, for example, nucleic acids comprising one or more novel polymorphisms in the FRZB gene and/or nucleic acids useful for detecting one or more FRZB polymorphisms. Accordingly, one embodiment of the invention is an isolated nucleic acid molecule comprising a portion of the FRZB gene, its complement, and/or a variant thereof. Preferably said variant comprises at least one of the polymorphisms identified herein. Even more preferably, said variant comprises at least one of the polymorphisms identified herein to be associated with obesity. Alternatively, said variant comprises at least one of the polymorphisms

identified herein to be associated with osteoporosis. Thus, in one embodiment, the nucleic acid molecule comprises at least one of the FRZB polymorphisms provided in Table 1. In a further embodiment, the nucleic acid molecule comprises or consists of a primer and/or a probe specific to at least one of the polymorphisms identified in the

5 . FRZB gene (e.g., those identified herein to be associated with obesity and/or osteoporosis).

SNPS

In one aspect, the invention provides a method for detecting an individual's increased or

10   decreased risk for obesity by detecting the presence of one or more FRZB SNPs in a nucleic acid sample of the individual, wherein the presence of said SNP(s) indicates the individual's increased or decreased risk for obesity. In a further aspect, the invention provides a method for detecting an individual's increased or decreased risk for osteoporosis by detecting the presence of one or more FRZB SNPs in a nucleic acid

15   sample of the individual, wherein the presence of said SNP(s) indicates the individual's increased or decreased risk for osteoporosis. The SNPs can be any SNPs in the FRZB locus including SNPs in exons, introns and/or upstream and/or downstream regions. Examples of such SNPs include, but are not limited to, those provided in Table 1, below, and discussed in detail in the Examples. In one embodiment, the SNPs present in the

20   FRZB locus are identified by genotyping the FRZB SNPs.

Table 1: FRZB SNPs

FRZB SNP	Standard Name	Position in NT_005403	SNP Source	Pos. in Gene/Change
FRZB1_T2303723C	NT_005265.11_2303723	NT_005403_33933138	rs6433992	Intron 1/T-C
FRZB_C18679T	NT_005100.3_18679	NT005403_33917116	rs288330	Intron 2/C-T
FRZB_G19524A	NT_005100.3_19524	NT005403_33916271	rs2242070	Intron 3/G-A
FRZB_T19575G	NT_005100.3_19575	NT005403_33916220	RMS SNP	Intron 3/T-G
FRZB_T22242A	NT_005100.3_22242	NT005403_33913553	rs288327	Intron 3/T-A
PRZB_G23043A	NT_005100.3_23043	NT005403_33912752	rs288326	Exon 4/G-A->Arg200Trp
FRZB_G23415A	NT_005100.3_23415	NT005403_33912380	rs1561369	Intron 4/G-A
FRZB_T23549C	NT_005100.3_23549	NT005403_33912246	rs288325	Intron 4/T-C

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FRZB SNP	Standard Name	Position in NT_005403	SNP Source	Pos. in Gene/Change
FRZB_A24791G	NT_005100.3_24791	NT005403_33911004	rs288324	Intron 5/A-G
FRZB_C26794G	NT_005100.3_26794	NT005403_33909000	rs7775	Exon 6/C-G - >Arg324Gly
FRZB_G27014A	NT_005100.3_27014	NT005403_33908780	rs13009	3' UTR/G-A

In certain embodiments, the genotype of one FRZB SNP can be used to determine an
individual's risk for obesity and/or osteoporotic disease. In other embodiments, the
genotypes of a plurality of FRZB SNPs can be used. In other embodiments, certain
5   combinations of SNPs at either the same or different loci can be used. It is to be
understood that some of the SNPs related to an increased or decreased risk of obesity
may be the same as those related to either an increased or decreased risk of osteoporosis.

GENOTYPING METHODS

10   In the methods of the invention, the alleles present in a sample are identified by
identifying the nucleotide present at one or more of the polymorphic sites in a nucleic
acid sample of an individual. A number of methods are known in the art for identifying
the nucleotide present at polymorphic sites. The particular method used to identify the
genotype is not a critical aspect of the invention. Although considerations of
15   performance, cost, and convenience will make particular methods more desirable than
others, it will be clear that any method that can identify the nucleotide present will
provide the information needed to identify the genotype. Preferred genotyping methods
involve DNA sequencing, allele-specific amplification, or probe-based detection of
amplified nucleic acid.

20   FRZB alleles can be identified by DNA sequencing methods, such as the chain
termination method (Sanger et al., 1977, Proc. Natl. Acad. Sci., 74:5463-5467,
incorporated herein by reference), which are well known in the art. In one
embodiment, a portion of the gene encompassing the polymorphic site is amplified and
either cloned into a suitable plasmid and then sequenced, or sequenced directly. PCR-
25   based sequencing is described in U.S. Patent No. 5,075,216; Brow, in PCR Protocols,
1990, (Innis et al., eds., Academic Press, San Diego), chapter 24; and Gyllensten, in PCR
Technology, 1989 (Erlich, ed., Stockton Press, New York), chapter 5; each incorporated
herein by reference. Typically, sequencing is performed using an automated DNA

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sequencer, which are commercially available from, for example, PE Biosystems (Foster City, CA), Pharmacia (Piscataway, NJ), Genomyx Corp. (Foster City, CA), LI-COR Biotech (Lincoln, NE), GeneSys technologies (Sauk City, WI), and Visible Genetics, Inc. (Toronto, Canada).

5   FRZB alleles can also be identified using amplification-based genotyping methods. Various nucleic acid amplification methods known in the art can be used in to detect nucleotide changes in a target nucleic acid. A preferred method is the polymerase chain reaction (PCR), which is now well known in the art, and described in U.S. Patent Nos. 4,683,195; 4,683,202; and 4,965,188; each incorporated herein by reference. Examples of

10   the numerous articles published describing methods and applications of PCR are found in PCR Applications, 1999, (Innis *et al.*, eds., Academic Press, San Diego), PCR Strategies, 1995, (Innis *et al.*, eds., Academic Press, San Diego); PCR Protocols, 1990, (Innis *et al.*, eds., Academic Press, San Diego); and PCR Technology, 1989, (Erlich, ed., Stockton Press, New York); each incorporated herein by reference. Commercial

15   vendors, such as PE Biosystems (Foster City, CA) market PCR reagents and publish PCR protocols.

Other suitable amplification methods include the ligase chain reaction (Wu and Wallace, 1988, *Genomics* 4:560-569); the strand displacement assay (Walker *et al.*, 1992, *Proc. Natl. Acad. Sci. USA* 89:392-396, Walker *et al.* 1992, *Nucleic Acids Res.* 20:1691-

20   1696, and U.S. Patent No. 5,455,166); and several transcription-based amplification systems, including the methods described in U.S. Patent Nos. 5,437,990; 5,409,818; and 5,399,491; the transcription amplification system (TAS) (Kwoh *et al.*, 1989, *Proc. Natl. Acad. Sci. USA*, 86:1173-1177); and self-sustained sequence replication (3SR) (Guatelli *et al.*, 1990, *Proc. Natl. Acad. Sci. USA*, 87:1874-1878 and WO 92/08800); each

25   incorporated herein by reference. Alternatively, methods that amplify the probe to detectable levels can be used, such as Qß-replicase amplification (Kramer *et al.*, 1989, *Nature*, 339:401-402, and Lomeli *et al.*, 1989, *Clin. Chem.*, 35:1826-1831, both of which are incorporated herein by reference). A review of known amplification methods is provided in Abramson *et al.*, 1993, *Current Opinion in Biotechnology*, 4:41-47,

30   incorporated herein by reference.

Genotyping can also be carried out by detecting and analyzing FRZB mRNA under conditions when both maternal and paternal chromosomes are transcribed. Amplification of RNA can be carried out by first reverse-transcribing the target RNA using, for example, a viral reverse transcriptase, and then amplifying the resulting

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cDNA, or using a combined high-temperature reverse-transcription-polymerase chain reaction (RT-PCR), as described in U.S. Patent Nos. 5,310,652; 5,322,770; 5,561,058; 5,641,864; and 5,693,517; each incorporated herein by reference (see also Myers and Sigua, 1995, in PCR Strategies, *supra*, chapter 5).

5   FRZB alleles can also be identified using allele-specific amplification or primer extension methods, which are based on the inhibitory effect of a terminal primer mismatch on the ability of a DNA polymerase to extend the primer. To detect an allele sequence using an allele-specific amplification or extension-based method, a primer complementary to the FRZB genes is chosen such that the 3' terminal nucleotide hybridizes at the polymorphic

10   position. In the presence of the allele to be identified, the primer matches the target sequence at the 3' terminus and primer is extended. In the presence of only the other allele, the primer has a 3' mismatch relative to the target sequence and primer extension is either eliminated or significantly reduced. Allele-specific amplification- or extension-based methods are described in, for example, U.S. Patent Nos. 5,137,806; 5,595,890;

15   5,639,611; and U.S. Patent No. 4,851,331, each incorporated herein by reference.

Using allele-specific amplification-based genotyping, identification of the alleles requires only detection of the presence or absence of amplified target sequences. Methods for the detection of amplified target sequences are well known in the art. For example, gel electrophoresis (see Sambrook *et al.*, 1989, *infra*) and the probe hybridization assays

20   described above have been used widely to detect the presence of nucleic acids.

Allele-specific amplification-based methods of genotyping can facilitate the identification of haplotypes, as described in the examples. Essentially, the allele-specific amplification is used to amplify a region encompassing multiple polymorphic sites from only one of the two alleles in a heterozygous sample. The SNP variants present within

25   the amplified sequence are then identified, such as by probe hybridization or sequencing.

An alternative probe-less method, referred to herein as a kinetic-PCR method, in which the generation of amplified nucleic acid is detected by monitoring the increase in the total amount of double-stranded DNA in the reaction mixture, is described in Higuchi

30   *et al.*, 1992, *Bio/Technology*, 10:413-417; Higuchi *et al.*, 1993, *Bio/Technology*, 11:1026-1030; Higuchi and Watson, in PCR Applications, supra, Chapter 16; U.S. Patent Nos. 5,994,056 and 6,171,785; and European Patent Publication Nos. 487,218 and 512,334, each incorporated herein by reference. The detection of double-stranded target DNA

relies on the increased fluorescence that DNA-binding dyes, such as ethidium bromide or SYBR™ Green, exhibit when bound to double-stranded DNA. The increase of double-stranded DNA resulting from the synthesis of target sequences results in an increase in the amount of dye bound to double-stranded DNA and a concomitant

5   detectable increase in fluorescence. For genotyping using the kinetic-PCR methods, amplification reactions are carried out using a pair of primers specific for one of the alleles, such that each amplification can indicate the presence of a particular allele. For example, by performing two amplifications, one using primers specific for the wild-type allele and one using primers specific for the mutant allele, the genotype of the sample

10   with respect to that SNP can be determined. Similarly, by carrying out four amplifications, each with one of the possible pairs possible using allele specific primers for both the upstream and downstream primers, the genotype of the sample with respect to two SNPs can be determined. This gives haplotype information for a pair of SNPs.

       Alleles can be also identified using probe-based methods, which rely on the difference in
15   stability of hybridization duplexes formed between a probe and its corresponding target sequence comprising a FRZB allele. Under sufficiently stringent hybridization conditions, stable duplexes are formed only between a probe and its target allele sequence and not other allele sequences. The presence of stable hybridization duplexes can be detected by any of a number of well known methods. In general, it is preferable

20   to amplify a nucleic acid encompassing a polymorphic site of interest prior to hybridization in order to facilitate detection. However, this is not necessary if sufficient nucleic acid can be obtained without amplification.

       A probe suitable for use in the probe-based methods of the invention, which contains a hybridizing region either substantially complementary or exactly complementary to a
25   target region of the FRZB gene or the complement thereof, wherein the target region encompasses the polymorphic site, and exactly complementary to one of the two allele sequences at the polymorphic site, can be selected using the guidance provided herein and well known in the art. Similarly, suitable hybridization conditions (e.g., stringent hybridization conditions), which depend on the exact size and sequence of the probe,
30   can be selected empirically using the guidance provided herein and well known in the art (see, e.g., Nucleic Acid Hybridization (B.D. Hames and S.J. Higgins. eds., 1984) and Sambrook et al., Molecular Cloning - A Laboratory Manual (3rd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2000). The use of oligonucleotide probes to detect nucleotide variations including single base pair
35   differences in sequence is described in, for example, Conner *et al.*, 1983, *Proc. Natl.*

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Acad. Sci. USA, 80:278-282, and U.S. Patent Nos. 5,468,613 and 5,604,099, each incorporated herein by reference.

In some embodiments of the probe-based methods for determining the FRZB genotypes, multiple nucleic acid sequences from the FRZB genes which encompass the

5   polymorphic sites are amplified and hybridized to a set of probes under sufficiently stringent hybridization conditions. The alleles present are inferred from the pattern of binding of the probes to the amplified target sequences. In this embodiment, amplification is carried out in order to provide sufficient nucleic acid for analysis by probe hybridization. Thus, primers are designed such that regions of the FRZB genes

10   encompassing the polymorphic sites are amplified regardless of the allele present in the sample. Allele-independent amplification is achieved using primers which hybridize to conserved regions of the FRZB genes. The FRZB genes contain many invariant or monomorphic regions, and suitable allele-independent primers can be selected routinely from SEQ ID NO:1 or GenBank accession numbers NT_005100.3, NT_005265, or

15   NT_005403.

Suitable assay formats for detecting hybrids formed between probes and target nucleic acid sequences in a sample are known in the art and include the immobilized target (dot-blot) format and immobilized probe (reverse dot-blot or line-blot) assay formats. Dot blot and reverse dot blot assay formats are described in U.S. Patent Nos. 5,310,893;

20   5,451,512; 5,468,613; and 5,604,099; each incorporated herein by reference.

In a dot-blot format, amplified target DNA is immobilized on a solid support, such as a nylon membrane. The membrane-target complex is incubated with labeled probe under suitable hybridization conditions, unhybridized probe is removed by washing under suitably stringent conditions, and the membrane is monitored for the presence of bound

25   probe. A preferred dot-blot detection assay is described in the examples.

In the reverse dot-blot (or line-blot) format, the probes are immobilized on a solid support, such as a nylon membrane or a microtiter plate. The target DNA is labeled, typically during amplification by the incorporation of labeled primers. One or both of the primers can be labeled. The membrane-probe complex is incubated with the labeled

30   amplified target DNA under suitable hybridization conditions, unhybridized target DNA is removed by washing under suitably stringent conditions, and the membrane is monitored for the presence of bound target DNA. A preferred reverse line-blot detection assay is described in the examples.

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Probe-based genotyping can be carried out using a 5'-nuclease assay, as described in U.S.
Patent Nos. 5,210,015; 5,487,972; and 5,804,375; and Holland *et al.*, 1988, *Proc. Natl.*
Acad. Sci. USA, 88:7276-7280, each incorporated herein by reference. In the 5'-
nuclease assay, labeled detection probes that hybridize within the amplified region are

5   added during the amplification reaction mixture. The probes are modified so as to
prevent the probes from acting as primers for DNA synthesis. The amplification is
· carried out using a DNA polymerase that possesses 5' to 3' exonuclease activity, *e.g.*, *Tth*
DNA polymerase. During each synthesis step of the amplification, any probe which
hybridizes to the target nucleic acid downstream from the primer being extended is

10   degraded by the 5' to 3' exonuclease activity of the DNA polymerase. Thus, the
synthesis of a new target strand also results in the degradation of a probe, and the
accumulation of degradation product provides a measure of the synthesis of target
sequences.

Any method suitable for detecting degradation product can be used in the 5'-nuclease

15   assay. In a preferred method, the detection probes are labeled with two fluorescent dyes,
one of which is capable of quenching the fluorescence of the other dye. The dyes are
attached to the probe, preferably one attached to the 5' terminus and the other is
attached to an internal site, such that quenching occurs when the probe is in an
unhybridized state and such that cleavage of the probe by the 5' to 3' exonuclease activity

20   of the DNA polymerase occurs in between the two dyes. Amplification results in
cleavage of the probe between the dyes with a concomitant elimination of quenching
and an increase in the fluorescence observable from the initially quenched dye. The
accumulation of degradation product is monitored by measuring the increase in
reaction fluorescence. U.S. Patent Nos. 5,491,063 and 5,571,673, both incorporated

25   herein by reference, describe alternative methods for detecting the degradation of probe
which occurs concomitant with amplification.

The 5'-nuclease assay can be used with allele-specific amplification primers such that the
probe is used only to detect the presence of amplified product. Such an assay is carried
out as described for the kinetic-PCR-based methods described above. Alternatively, the

30   5'-nuclease assay can be used with a target-specific probe.

Examples of other techniques that can be used for probe-based genotyping include, but
are not limited to, Amplifluor™, Dye Binding-Intercalation, Fluorescence Resonance
Energy Transfer (FRET), Hybridization Signal Amplification Method (HSAM), HYB
Probes™, Invader/Cleavase Technology (Invader/CFLP™), Molecular Beacons™,

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Origen™, DNA-Based Ramification Amplification (RAM™), Rolling circle amplification (RCA™), Scorpions™, Strand displacement amplification (SDA).

The assay formats described above typically utilize labeled oligonucleotides to facilitate detection of the hybrid duplexes. Oligonucleotides can be labeled by incorporating a
5   label detectable by spectroscopic, photochemical, biochemical, immunochemical, radiological, radiochemical or chemical means. Useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes (as commonly used in ELISAs), biotin, or haptens and proteins for which antisera or monoclonal antibodies are available. Labeled oligonucleotides of the invention can be synthesized and labeled using the techniques
10   described above for synthesizing oligonucleotides. For example, a dot-blot assay can be carried out using probes labeled with biotin, as described in Levenson *et al.*, 1989, in *PCR Protocols: A Guide to Methods and Applications* (Innis *et al.*, eds., Academic Press. San Diego), pages 99-112, incorporated herein by reference. Following hybridization of the immobilized target DNA with the biotinylated probes under sequence-specific
15   conditions, probes which remain bound are detected by first binding the biotin to avidin-horseradish peroxidase (A-HRP) or streptavidin-horseradish peroxidase (SA-HRP), which is then detected by carrying out a reaction in which the HRP catalyzes a color change of a chromogen.

Whatever the method for determining which oligonucleotides of the invention
20   selectively hybridize to FRZB allelic sequences in a sample, the central feature of the typing method involves the identification of the FRZB alleles present in the sample by detecting the variant sequences present. Further details on genotyping of SNPs are available in the literature; see, e.g., Lindblad-Toh *et al.*, 2000, *Nature Genetics* 24:381-386; Plant Genotyping: The DNA Fingerprinting of Plants, 2001, CABI Publishing;
25   Syvanen, 2001, *Nat. Rev. Genet.* 2:930-942; Kuklin *et al.*, 1998, *Genetic Testing* 1: 201-206; Gut, 2001, *Hum. Mutat.* 17:475-492; Ahmadian *et al.*, 2000, *Anal. Biochem.* 280:103-110; Useche *et al.*, 2001, *Genome Inform Ser Workshop Genome Inform* 12:194-203; Pastinen *et al.*, 2000, *Genome Res.* 10:1031-1042; Hacia, 1999, *Nature Genet.* 22:164-167; and Chen *et al.*, 2000, *Genome Res.* 10:549-557.

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OTHER MARKERS

Other genetic markers and methods of detecting sequence polymorphisms are known in
the art and can be applied to the practice of the present invention, including, but not
5   limited to, restriction fragment length polymorphisms (RFLPs), random amplified
polymorphic DNA (RAPD), arbitrary fragment length polymorphisms (AFLPs), simple
sequence repeats (SSRs), single-stranded conformation polymorphisms (SSCPs), and
amplified variable sequences. Discovery, detection, and genotyping of these and other
types of genetic markers has been well described in the literature. See, e.g., Orita *et al.*,
10 1989, *Proc. Natl. Acad. Sci. USA* 86:2766-2770; USPN 6,399,855; Henry, ed., 2001, Plant
Genotyping. The DNA Fingerprinting of Plants Wallingford: CABI Publishing; Phillips
and Vasil, eds., 2001, DNA-based Markers in Plants Dordrecht: Kluwer Academic
Publishers; Pejic *et al.*, 1998, *Theor. App. Genet.* 97:1248-1255; Bhattramakki *et al.*, 2002,
Plant Mol. Biol. 48:539-47; Nickerson *et al.*, 1997, *Nucleic Acids Res.* 25:2745-2751;
15 Underhill *et al.*, 1997, *Genome Res.* 7:996-1005; Shi, 2001, *Clin. Chem.* 47:164-172;
Kwok, 2000, *Pharmacogenomics* 1:95-100; Rafalski *et al.*, 2002, *Cell Mol Biol Lett* 7:471-5;
Ching and Rafalski, 2002, *Cell Mol Biol Lett.* 7:803-10; Powell *et al.*, 1996, *Mol. Breeding*
2:225-238; Vos *et al.*, 1995, *Nucl. Acids Res.* 23:4407; Becker *et al.*, 1995, *Mol. Gen. Genet.*
249:65; Meksem *et al.*, 1995, *Mol. Gen. Genet.* 249:74; Huys *et al.*, 1996, *Int'l J. Systematic*
20 *Bacteriol.* 46:572; Jacob *et al.*, 1991, *Cell* 67:213; Taramino and Tingey, 1996, *Genome*
39:277-287; Condit and Hubbell, 1991, *Genome* 34:66; and Zietkiewicz *et al.*, 1994,
Genomics 20:176-83.

ASSOCIATION ANALYSIS

25 Evaluation of the candidate gene FRZB for association with various phenotypes
pertaining to obesity and osteoporosis is described in the Examples. In addition, design
and execution of various types of association studies have been described in the art; see,
e.g., Rao and Province, eds., 2001, Advances in Genetics volume 42, Genetic Dissection
of Complex Traits; Balding *et al.*, eds., 2001, Handbook of Statistical Genetics, John
30 Wiley and Sons Ltd.; Borecki and Suarez, 2001, *Adv Genet* 42:45-66; Cardon and Bell,
2001, *Nat Rev Genet* 2:91-99; and Risch, 2000, *Nature* 405:847-856. Association studies
have been used both to evaluate candidate genes for association with a phenotypic trait
(e.g., Thornsberry *et al.*, 2001, *Nature Genetics* 28:286-289) and to perform whole
genome scans to identify genes that contribute to phenotypic variation.

KITS

The invention also relates to a kit comprising a container unit and components for
practicing the present method. A kit can contain oligonucleotide probes specific for
5   FRZB alleles as well as instructions for their use to determine risk for obesity and/or
osteoporosis. In some cases, a kit may comprise detection probes fixed to an
appropriate support membrane. The kit can also contain amplification primers for
amplifying regions of the FRZB locus encompassing the polymorphic sites, as such
primers are useful in the preferred embodiment of the invention. Alternatively, useful
10   kits can contain a set of primers comprising an allele-specific primer for the specific
amplification of FRZB alleles. Other optional components of the kits include additional
reagents used in the genotyping methods as described herein. For example, a kit
additionally can contain an agent to catalyze the synthesis of primer extension products,
substrate nucleoside triphosphates, reagents for labeling and/or detecting nucleic acid
15   (for example, an avidin-enzyme conjugate and enzyme substrate and chromogen if the
label is biotin) and appropriate buffers for amplification or hybridization reactions.

One aspect of the invention provides kits for detecting presence of a first predisposing or
protective polymorphism in an FRZB gene, e.g., in a nucleic acid sample of an individual
whose risk for osteoporosis and/or obesity is being assessed. Thus, one general class of
20   embodiments provides a kit including one or more first oligonucleotides capable of
detecting the first polymorphism and instructions for detecting the first polymorphism
with the one or more first oligonucleotides and for correlating said detection to the
individual's risk for osteoporosis and/or obesity, packaged in one or more containers.

Essentially all of the features noted for the method embodiments above apply to this
25   embodiment as well, as relevant. For example, in a preferred class of embodiments, the
first polymorphism is a single nucleotide polymorphism, e.g., a SNP selected from the
group consisting of: the T allele of T2303723C, the C allele of T2303723C, the C allele of
C18679T, the T allele of C18679T, the G allele of G19524A, the A allele of G19524A, the
T allele of T22242A, the A allele of T22242A, the A allele of A24791G, the G allele of
30   A24791G, the C allele of C26794G, the G allele of C26794G, the G allele of G27014A,
and the A allele of G27014A. Other potential SNPs include, but are not limited to, either
allele of T19575G, G23043A, G23415A, and T23549C.

In one aspect, the kit can be used to detect the presence of the first polymorphism by
hybridization of a nucleic acid probe to the polymorphism. Thus, in one class of

embodiments, the one or more first oligonucleotides comprise at least one probe. In certain embodiments, the first oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the first polymorphism. In one class of embodiments, the first polymorphism is a first single nucleotide polymorphism

5 comprising a first nucleotide at a first nucleotide position. In this class of embodiments, under stringent conditions, the first oligonucleotide hybridizes to a region of the FRZB gene comprising the first single nucleotide polymorphism with a signal to noise ratio that is at least 2x (e.g., at least 5x or at least 10x) the signal to noise ratio at which the first oligonucleotide hybridizes to the region of the FRZB gene comprising a second

10 nucleotide at the first nucleotide position. The first oligonucleotide is typically fully complementary to the region of the FRZB gene comprising the first polymorphism, and typically comprises at least about 10 contiguous nucleotides complementary to the FRZB gene.

To facilitate detection of the polymorphism (e.g., through detection of hybridization

15 between the one or more first oligonucleotides and a nucleic acid comprising the polymorphism), for example, the one or more first oligonucleotides optionally comprise a label, e.g., an isotopic, fluorescent, fluorogenic, luminescent or colorimetric label. In some embodiments, the label itself directly produces a detectable signal (e.g., a fluorescent label). In other embodiments, the kit also includes a reagent that detects the

20 label (e.g., an enzyme that cleaves a colorimetric label, a binding moiety, or the like).

In one aspect, the one or more first oligonucleotides comprise one or more primers. The primer(s) can be used to detect the polymorphism, e.g., in an allele-specific amplification or extension reaction. For example, in one class of embodiments, the first polymorphism is a first single nucleotide polymorphism comprising a first nucleotide at

25 a first nucleotide position, and the 3' nucleotide of one of the one or more first oligonucleotides is complementary to the first nucleotide.

The primer(s) can be used to amplify a region of FRZB comprising the polymorphism, e.g., for subsequent detection of the polymorphism by hybridization, sequencing, or the like. In one class of embodiments, the one or more first oligonucleotides comprise

30 amplification primers, wherein the amplification primers amplify a nucleic acid sequence comprising the first polymorphism. In a related class of embodiments, the one or more first oligonucleotides comprise sequencing primers that flank the first polymorphism.

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The one or more first oligonucleotides are optionally immobilized on a substrate. The substrate can be, for example, a planar substrate or a beaded substrate. The oligonucleotide(s) can be arranged in an array of other oligonucleotides used to detect other polymorphisms, e.g., other polymorphisms in FRZB.

5   The kit can optionally be used to detect more than one polymorphism (simultaneously or sequentially). Thus, in one class of embodiments, the kit also includes one or more second oligonucleotides capable of detecting a second polymorphism (and optionally third, fourth, fifth, etc. oligonucleotides capable of detecting third, fourth, fifth, etc. polymorphisms). The second polymorphism can be at the same polymorphic site as the

0   first or at a different polymorphic site (in FRZB or a different gene), and can be protective or predisposing.

ARRAYS AND SYSTEMS

The invention also relates to an array, a support with immobilized oligonucleotides

5   useful for practicing the present method. A useful array can contain oligonucleotide probes specific for FRZB alleles or certain combinations of FRZB alleles. The oligonucleotides can be immobilized on a substrate, e.g., a membrane or glass. The oligonucleotides can, but need not, be labeled. In some embodiments, the array can be a micro-array. In some embodiments, the array can comprise one or more

!0   oligonucleotides used to detect the presence of two or more FRZB alleles or certain combinations of FRZB alleles.

One general class of embodiments provides arrays for detecting presence of one or more predisposing and/or protective polymorphisms in an FRZB gene, e.g., in a nucleic acid sample of an individual whose risk for osteoporosis and/or obesity is being assessed. In

!5   one class of embodiments, the array comprises a substrate and a plurality of oligonucleotides, each of which oligonucleotides hybridizes to a region of the FRZB gene comprising at least one of the polymorphisms. The hybridization detects the presence of the polymorphism, and this detection provides an indication of the individual's risk for osteoporosis and/or obesity. Typically, the array is used for detecting the presence of a

!0   plurality of polymorphisms, e.g., multiple alleles at a single polymorphic site and/or different polymorphic sites.

Essentially all of the features noted for the method and kit embodiments above apply to this embodiment as well, as relevant. For example, the one or more polymorphisms

preferably comprise one or more single nucleotide polymorphisms. For example, at least one of the one or more polymorphisms can be selected from the group consisting of: the T allele of T2303723C, the C allele of T2303723C, the C allele of C18679T, the T allele of C18679T, the G allele of G19524A, the A allele of G19524A, the T allele of

5   T22242A, the A allele of T22242A, the A allele of A24791G, the G allele of A24791G, the C allele of C26794G, the G allele of C26794G, the G allele of G27014A, and the A allele of G27014A. Other potential SNPs include, but are not limited to, either allele of T19575G, G23043A, G23415A, and T23549C.

In one class of embodiments in which the array can be used to detect presence of one or

.0   more SNPs, each of the oligonucleotides in the array hybridizes under stringent conditions to a region of the FRZB gene comprising one of the single nucleotide polymorphisms with a signal to noise ratio that is at least 2x (e.g., at least 5x or at least 10x) that at which the oligonucleotide hybridizes to a region of the FRZB gene comprising any of the remaining single nucleotide polymorphisms. Typically, one

15   oligonucleotide is used to detect one SNP; that is, each of the oligonucleotides typically hybridizes to a distinct single nucleotide polymorphism.

As noted, the plurality of oligonucleotides are immobilized on a substrate, e.g., a planar substrate, a membrane, a glass slide, or the like. Typically, each of the plurality of oligonucleotides is immobilized at a known, pre-determined position on the substrate.

20   To facilitate detection of polymorphisms by specific hybridization with the oligonucleotides, each of the plurality of oligonucleotides is typically fully complementary to a region of the FRZB gene comprising one of the polymorphisms, and each of the plurality of oligonucleotides typically comprises at least about 10 contiguous nucleotides complementary to the FRZB gene. Each of the plurality of

25   oligonucleotides optionally comprises a label, e.g., a label that facilitates detection of hybridization between the oligonucleotides and the polymorphisms.

The array is optionally part of a system. Thus, one class of embodiments provides a system comprising an array of the invention and system instructions that correlate the detection of the presence of one or more predisposing or protective polymorphisms to

30   the individual's risk for osteoporosis and/or obesity. Systems, e.g., digital systems, are described in greater detail below.

In an array on a substrate, each oligonucleotide is typically bound (e.g., electrostatically or covalently bound, directly or via a linker) to the substrate at a unique location.

Methods of making, using, and analyzing such arrays (e.g., microarrays) are well known
in the art. See, e.g., Wang et al., 1998, Science 280:1077-82; Lockhart and Winzeler,
2000, Nature 405:827-836; and Scherf et al., 2000, Nat Genet. 24:236-44. Arrays can be
formed (e.g., printed), for example, using commercially available instruments such as a
5   GMS 417 Arrayer (Affymetrix, Santa Clara, CA). Suitable solid supports are
commercially readily available. For example, a variety of membranes (e.g., nylon, PVDF,
and nitrocellulose membranes) are commercially available, e.g., from Sigma-Aldrich,
Inc. (www.sigmaaldrich.com). As another example, surface-modified and pre-coated
slides with a variety of surface chemistries are commercially available, e.g., from
10   TeleChem International (www.arrayit.com), Corning, Inc. (Corning, NY), or Greiner
Bio-One, Inc. (www.greinerbiooneinc.com). For example, silanated and silyated slides
with free amino and aldehyde groups, respectively, are available and permit covalent
coupling of molecules (e.g., oligos) to the slides. Slides with surface streptavidin are
available and can bind biotinylated oligos. In addition, services that produce arrays of
15   nucleic acids of the customer's choice are commercially available, e.g., from TeleChem
International (www.arrayit.com).

DIGITAL SYSTEMS

In general, various automated systems can be used to perform some or all of the method
20   steps as noted herein. In addition to practicing some or all of the method steps herein,
digital or analog systems, e.g., comprising a digital or analog computer, can also control
a variety of other functions such as a user viewable display (e.g., to permit viewing of
method results by a user) and/or control of output features.

For example, certain of the methods described above are optionally implemented via a
25   computer program or programs (e.g., that correlate detection of the presence of one or
more predisposing or protective polymorphisms to an individual's risk for osteoporosis
and/or obesity). Thus, the present invention provides digital systems, e.g., computers,
computer readable media, and/or integrated systems comprising instructions (e.g.,
embodied in appropriate software) for performing the methods herein. For example, a
30   digital system comprising instructions for correlating detection of the presence of one or
more predisposing or protective polymorphisms to an individual's risk for osteoporosis
and/or obesity, as described herein, is a feature of the invention. The digital system can
also include information (data) corresponding to individual genotypes for a set of
genetic markers, phenotypic values, and/or the like. The system can also aid in detection

of the one or more polymorphisms (e.g., by controlling a microarray scanner, or the like).

Standard desktop applications such as word processing software (e.g., Microsoft Word™ or Corel WordPerfect™) and/or database software (e.g., spreadsheet software such as

5   Microsoft Excel™, Corel Quattro Pro™, or database programs such as Microsoft Access™ or Paradox™) can be adapted to the present invention by inputting data which is loaded into the memory of a digital system, and performing an operation as noted herein on the data. For example, systems can include the foregoing software having the appropriate genotypic information, associations between phenotype and genotype, etc.,

10   e.g., used in conjunction with a user interface (e.g., a GUI in a standard operating system such as a Windows, Macintosh or LINUX system) to perform any analysis noted herein, or simply to acquire data (e.g., in a spreadsheet) to be used in the methods herein.

Systems typically include, e.g., a digital computer with software for performing association analysis and/or risk prediction, as well as data sets entered into the software

15   system comprising genotypes for a set of genetic markers, phenotypic values, and/or the like. The computer can be, *e.g.*, a PC (Intel x86 or Pentium chip- compatible DOS,™ OS2,™ WINDOWS,™ WINDOWS NT,™ WINDOWS95,™ WINDOWS98,™ LINUX, Apple-compatible, MACINTOSH™ compatible, Power PC compatible, or a UNIX compatible (*e.g.*, SUN™ work station) machine) or other commercially common

20   computer which is known to one of skill. Software for performing association analysis and/or risk prediction can be constructed by one of skill using a standard programming language such as Visualbasic, Fortran, Basic, Java, or the like, according to the methods herein.

Any system controller or computer optionally includes a monitor which can include,

25   e.g., a cathode ray tube ("CRT") display, a flat panel display (*e.g.*, active matrix liquid crystal display, liquid crystal display), or others. Computer circuitry is often placed in a box which includes numerous integrated circuit chips, such as a microprocessor, memory, interface circuits, and others. The box also optionally includes a hard disk drive, a floppy disk drive, a high capacity removable drive such as a writeable CD-ROM,

30   and other common peripheral elements. Inputting devices such as a keyboard or mouse optionally provide for input from a user and for user selection of genetic marker genotype, phenotypic value, or the like in the relevant computer system.

The computer typically includes appropriate software for receiving user instructions, either in the form of user input into a set of parameter fields, e.g., in a GUI, or in the form of preprogrammed instructions, e.g., preprogrammed for a variety of different specific operations. The software then converts these instructions to appropriate

5   language for instructing the system to carry out any desired operation. For example, in addition to performing risk prediction, a digital system can control equipment for detecting polymorphisms according to the relevant method herein.

The invention can also be embodied within the circuitry of an application specific integrated circuit (ASIC) or programmable logic device (PLD). In such a case, the

10   invention is embodied in a computer readable descriptor language that can be used to create an ASIC or PLD. The invention can also be embodied within the circuitry or logic processors of a variety of other digital apparatus, such as PDAs, laptop computer systems, displays, image editing equipment, etc.

MOLECULAR BIOLOGICAL TECHNIQUES

15

Conventional techniques of molecular biology and nucleic acid chemistry, which are within the skill of the art, are fully explained in the literature. See, for example, Sambrook et al., 1989, Molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York; Oligonucleotide Synthesis (M.J. Gait, ed.,

20   1984); Nucleic Acid Hybridization (B.D. Hames and S.J. Higgins. eds., 1984); the series, Methods in Enzymology (Academic Press, Inc.); the series Current Protocols in Human Genetics (Dracopoli et al., eds., 1984 with quarterly updates, John Wiley & Sons, Inc.); Berger and Kimmel, Guide to Molecular Cloning Techniques, Methods in Enzymology volume 152 Academic Press, Inc., San Diego, CA; Sambrook et al., Molecular Cloning -

25   A Laboratory Manual (3rd Ed.), Vol. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 2000; Current Protocols in Molecular Biology, F.M. Ausubel et al., eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (supplemented through 2004); Freshney (1994) Culture of Animal Cells, a Manual of Basic Technique, third edition, Wiley- Liss, New York and the

30   references cited therein; Payne et al. (1992) Plant Cell and Tissue Culture in Liquid Systems John Wiley & Sons, Inc. New York, NY; Gamborg and Phillips (Eds.) (1995) Plant Cell, Tissue and Organ Culture; Fundamental Methods Springer Lab Manual, Springer-Verlag (Berlin Heidelberg New York); and Atlas and Parks (Eds.) The Handbook of Microbiological Media (1993) CRC Press, Boca Raton, FL, all of which are

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incorporated herein by reference. All patents, patent applications, and publications mentioned herein, both supra and infra, are incorporated herein by reference.

EXAMPLES

5   The following examples are offered to illustrate, but not to limit, the claimed invention. It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and

10   purview of this application and scope of the appended claims.

Example 1: Genotyping Protocol: Allele-specific PCR of FRZB Alleles

This example describes a method of genotyping SNPs (and/or determining the frequency of alleles in pooled DNA samples) in the FRZB locus, e.g., SNPs that are associated with obesity and/or osteoporosis. The method used was basically that of

5   Germer, et al., *Genome Research*, 10:258-266 (2000). The method and primers were initially tested on cell lines listed in Table 2. The genotypes of these cell lines in FRZB had been previously determined by DNA sequencing for certain of the lines; other lines were genotyped by sequencing later, and some have not been sequenced to date.

Allele-specific primers and common primers were designed and tested. The allele specific PCR was performed using pairs of the primers listed in Table 2 (SEQ ID NOS:3-

0   29). All primers are shown in the 5' to 3' orientation. In addition to the primers, the control genotype of each cell line control for particular SNPs used in the test assays are also shown in Table 2.

Table 2: Allele-specific Assay Information

SNP	Cell Line Control	Control Genotype	Primer	Allele	Primer Sequences (SEQ ID NO:)
FRZB_C18679T	GM15890	A1/A1	AS1	C	ATAGTAGGAGGAGTACTGTGTCG (SEQ ID NO:3)
FRZB_C18679T	GM15892	A1/A2	AS2	T	AATAGTAGGAGGAGTACTGTGTCA (SEQ ID NO:4)
FRZB_C18679T	GM15891	A2/A2	Common		CCCTGTGGACTATCACCTAATGTT (SEQ ID NO:5)

SNP	Cell Line Control	Control Genotype	Primer	Allele	Primer Sequences (SEQ ID NO:)
FRZB_G19524A	GM15890	A1/A1	AS1	G	ATAGGCCATCAGTTGTGC (SEQ ID NO:6)
FRZB_G19524A	GM15208	A1/A2	AS2	A	CATAGGCCATCAGTTGTGT (SEQ ID NO:7)
FRZB_G19524A	GM15206	A2/A2	Common		TCAAAGTGCTCTGCTGTTCAC (SEQ ID NO:8)
FRZB_T22242A	GM15207	A1/A1	AS1	T	GATCTTATTTCCTCCATCTGCT (SEQ ID NO:9)
FRZB_T22242A	GM15889	A1/A2	AS2	A	ATCTTATTTCCTCCATCTGCA (SEQ ID NO:10)
FRZB_T22242A	GM15206	A2/A2	Common		CGTGAGGGAAAGGAATGTTG (SEQ ID NO:11)
FRZB_G23043A	GM15888	A1/A1	AS1	G	TTGTCTTTTATCCCAGTCATTC (SEQ ID NO:12)
FRZB_G23043A	GM15890	A1/A2	AS2	A	TTGTCTTTTATCCCAGTCATTT (SEQ ID NO:13)
FRZB_G23043A	GM13626	A1/A2	Common		CATCATGGCACTTAGTCTTTATCTC (SEQ ID NO:14)
FRZB_G23415A	GM15888	A1/A1	AS1	G	AAAAATGTAAACCTATAAACTACACG (SEQ ID NO:15)
FRZB_G23415A	GM15890	A1/A2	AS2	A	GAAAAATGTAAACCTATAAACTACACA (SEQ ID NO:16)
FRZB_G23415A	GM13626	A1/A2	Common		TCTTGATTTCATATATGGAATGGGT (SEQ ID NO:17)
FRZB_T23549C	GM15204	A1/A1	AS1	T	ACAGTACTTGAACAAGAAAGACTTAT (SEQ ID NO:18)
FRZB_T23549C	GM15889	A1/A2	AS2	C	ACAGTACTTGAACAAGAAAGACTTAC (SEQ ID NO:19)
FRZB_T23549C	NA14700	A1/A2	Common		ACTGTTCTAAATCTTAGCTGTCCTATTC (SEQ ID NO:20)
FRZB_G24791A	GM10347	A1/A1	AS1	G	CTCCCTTTTGACAAATCTACTG (SEQ ID NO:21)
FRZB_G24791A	GM11235	A1/A2	AS2	A	TCTCCCTTTTGACAAATCTACTA (SEQ ID NO:22)
FRZB_G24791A	GM13625	A2/A2	Common		GAAACTACCCTCCAGTAAGTTCTTC (SEQ ID

SNP	Cell Line Control	Control Genotype	Primer	Allele	Primer Sequences (SEQ ID NO:)
					NO:23)
FRZB_C26794G	GM11318	A1/A1	AS1	C	TTCGGGATTTAGTTGCG (SEQ ID NO:24)
FRZB_C26794G	GM10873	A1/A2	AS2	G	TTCGGGATTTAGTTGCC (SEQ ID NO:25)
FRZB_C26794G	NA14683	A2/A2	Common		GTCTGGCAGGAACTCGAACC (SEQ ID NO:26)
FRZB_G27014A	GM05045	A1/A1	AS1	G	TGGGGGCAGACTCTTAAG (SEQ ID NO:27)
FRZB_G27014A	GM10873	A1/A2	AS2	A	TGGGGGCAGACTCTTAAA(SEQ ID NO:28)
FRZB_G27014A	NA14683	A1/A2	Common		CATGATTAGTGAAATAGAAAACTCACA (SEQ ID NO:29)
FRZB_T19575G	GM10347	A1/A1	AS1	T	GACTGAAGAAGTCAAGTTTGAGT (SEQ ID NO:30)
FRZB_T19575G	GM04340	A1/A2	AS2	G	ACTGAAGAAGTCAAGTTTGAGG (SEQ ID NO:31)
FRZB_T19575G	GM13626	A1/A2	Common		TGAACAGCAGAGCACTTTGAT (SEQ ID NO:32)
FRZB_T2303723	GM12548	A1/A1	AS1	T	GTCGGCATTCTTATCATTCA (SEQ ID NO:33)
FRZB_T2303723 C	GM14663	A1/A2	AS2	C	CGGCATTCTTATCATTCG (SEQ ID NO:34)
FRZB_T2303723 C	GM14667	A2/A2	Common		AATAAGTCTCATCCATACTCAACCC (SEQ ID NO:35)

The PCR amplification was carried out in a total reaction volume of 50 µl containing the following reagents:

3.5 ng purified human genomic DNA

0.2 µM each primer (one common primer and one allele-specific primer)

50 µM each dATP, dCTP, dGTP

25 µM each dTTP

75 µM each dUTP

10 mM Tris-HCl, pH 8.3

3 mM MgCl$_2$

0.02U UNG (Uracil-n-glycosylase)

4% DMSO

2% glycerol

5   0.2X SYBR™ Green

12 units CEA2 Gold™ DNA polymerase*

* developed and manufactured by Hoffmann-La Roche. 0.5% glycerol added with CEA2 Gold. 1% DMSO added with SYBR Green.

The PCR was run on the GeneAmp 5700 Sequence Detection System (ABI) measuring

10   SYBR™ Green I (Molecular Probes, Eugene, OR, USA) fluorescence in realtime

(Higuchi, R. et al., 1993, *Biotechnology* 11:1026-30), as follows:

50°C for 2min

95°C for 12min

95°C for 20 sec

15   58°C for 20 sec

repeated for 45 cycles.

All of the primers shown in Table 2 used under these conditions resulted in the correct, known genotype for each SNP for the designated cell line. Thus, the genotyping assay is

20   usable for further analysis of the SNPs. The cell lines were used as positive controls in further analyses.

Example 2: Pooling Analysis for Identifying Disease Association with Known FRZB SNPs

Pooling analysis was used to facilitate the screening of candidate genes (e.g., the FRZB

25   gene) thought to be associated with osteoporosis and/or obesity. A large allele frequency difference between pools of DNA from patients and from controls is indicative of a possible involvement of a gene in these conditions. Instead of genotyping a very large number of samples, measuring the allele frequency of a single pool composed of equal amounts of those samples allows the rapid survey of a large number of candidate genes.

Eleven exemplary SNPs in the human FRZB gene are listed in Table 1. The allele frequencies of these SNPs were measured using allele-specific, quantitative PCR on pooled samples from the Study of Osteoporotic Fractures (SOF), using the method of Germer, et al., *Genome Research*, 10:258-266 (2000) as described in Example 1. The

5   Study of Osteoporotic Fractures (Kado, et al., *Arch. Intern. Med.* 159:1215-1220 (1999)) includes DNAs obtained from women age 65 or older exhibiting hip fractures, vertebral fractures, low bone mineral density (BMD) or high body mass index (BMI), as well as control samples. The study group comprises 1042 DNA samples altogether.

Allele Frequency Determination:

10

To measure a SNP allele frequency in a mixture of DNAs pooled from individual samples, equal aliquots of the pool are divided between two PCR reactions, each of which contains a primer pair specific to one or the other SNP allelic variant (e.g., one allele-specific primer and one common primer for each SNP, as described in Example

15   1). The specificity of the PCR amplification is conferred by placing the 3' end of one of the primers (the allele-specific primer) directly over and matching one or the other of the variant nucleotides. This specificity can be enhanced particularly by using the Stoffel fragment of Taq DNA polymerase or variants thereof. Ideally, only completely matched primers are extended, and only the matching allele is amplified. In practice, however,

20   there will typically be amplification of the mismatched allele, but this will occur much less efficiently such that many more amplification cycles are needed to generate detectable levels of product. Mismatch amplification is frequently delayed by >10 cycles when amplification is monitored on a cycle-by-cycle basis using fluorescent dsDNA binding dyes such as SYBR Green I. A delay of around six cycles is adequate for the

25   determination of allele frequencies of SNPs for which the frequency of the minor allele is greater than a few percent.

When the allele frequency is 50%, one expects that each of the two PCR amplifications will require the same number of cycles to produce the same fluorescent signal, assuming that both allele-specific primers amplify with equal efficiency. The number of cycles

30   before a reaction crosses a predetermined threshold, the C_t, can be fractional. When one allele is more frequent, the amplification of that allele will reach the threshold at an earlier cycle, that is, have a smaller C_t. The difference in C_t's between the two PCR reactions, the ΔC_t, is a measure of the bias and thus of the allele frequency. A one-cycle delay means that the ratio of the amount of one allele to the other is 1:2, a two-cycle

delay, 1:4, or in general, $1:2^{\Delta Ct}$. Converting a ratio to a frequency by adding the numerator to the denominator results in the equation

$$\text{Frequency of allele}_1 = 1/(2^{\Delta Ct} + 1),$$

Where $\Delta C_t = (C_t \text{ of allele}_1\text{-specific PCR}) - (C_t \text{ of allele}_2\text{-specific PCR}).$

5 Note that ΔC_t can be either positive or negative, depending on which specific PCR exhibits the lowest C_t. The "2" in the denominator is properly "1 + the initial replication efficiency". However, the initial replication efficiency is usually close to 100% so that "2" is an adequate approximation. The amplification efficiencies for the two allele-specific PCRs may differ slightly. As discussed in Germer *et al.* (*supra*), this

10 can be measured and compensated for by performing the assay on a DNA known to be heterozygous for the SNP of interest. The ΔC_t for this DNA should equal zero if the PCRs are equally efficient. Any deviation from zero indicates that they are not. This deviation can then be subtracted from all ΔC_t measurements to compensate for differential amplification efficiencies.

15 Each SNP except T2303723C was described by its position in the reference GenBank accession sequence NT_005100.3. This sequence (containing only 4 exons) was archived and replaced in GenBank with the more complete sequence containing 6 exons (NT_005265), which was in turn replaced by NT_005403. However, the original sequence for which the numbering was based (NT_005100.3) can be obtained from

20 GenBank, and the first 30,000 nucleotides of this sequence are presented as SEQ ID NO:1. Thus, for example, the second SNP listed in Table 1 is found at position 18679 of NT_005100.3 (and of SEQ ID NO:1), where a "T" nucleotide is present as the complement of nucleotide 18679 of SEQ ID NO:1. The common allele has a "C" nucleotide at this position. SNP T2303723C was described by its position in the

25 GenBank accession sequence NT_005265. The SNPs will be referred to by the SNP # (i.e., by nucleotide position in either SEQ ID NO:1 or NT_005265,as indicated above, although the nucleotide(s) indicated occupy either the indicated position or its complement, depending on the particular SNP; see Figure 1) in the subsequent text. Similarly, the SNPs can be located in any FRZB sequence by performing a sequence

30 alignment with the allele-specific primer sequences listed in Table 2. SNPs can also be unambiguously located in the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/index.html) through the SNP source number listed in Table 1.

The pooled samples were designated A through D (see, e.g., Table 3). The criterion for
pool A was hip fracture (any incident hip fracture since baseline, excluding prior hip
fracture since age 50). The criterion for pool B was vertebral fracture (incident vertebral
fracture by morphometry between baseline and visit 3). The criterion for pool C was

5   low BMD (bone mineral density, defined as having hip BMD T-score <-2.5). The
criteria for pool D (the control) were no fracture since age 50 and hip BMD Z-score
>1.285. The criterion for the High BMI pool was a body mass index in the highest 5% of
SOF participants and the criterion for the Low BMI pool was a body mass index in the
lowest 5% of SOF participants.

10 The pools involved combining equal amounts of DNAs from patients with each specific
criterion. Pool A included 275 patient samples; pool B contained 262 patient samples;
pool C included 276 patient samples; and pool D contained 278 patient samples. The
High BMI pool contained 141 samples and the Low BMI pool contained 82 samples.
The PCRs were performed in 4 replicates using the primers and protocol outlined in

15 Example 1 except that the PCRs contained 10 ng purified human genomic DNA and 2
uM Rox. The averages of the four replicates were calculated, and the allele frequencies
were then calculated following the method above and compared to the controls. A
change in allele frequency of greater than approximately 4-5% was considered
significant. The pooling results for each FRZB SNP are shown in Table 3 below.

20   Table 3: FRZB Pooling results

Pool	Average Allele 1 Frequency (%)						Change in Allele 1 Frequency (%)			
	A	B	C	D	Low BMI	High BMI	A-D	B-D	C-D	Low BMI-High BMI
FRZB_C18679T	66.38	61.81	69.06	63.79	71.02	65.75	2.59	-1.98	5.27	5.27
FRZB_G19524A	74.12	68.57	71.49	68.6	81.69	67.6	5.52	-0.03	2.89	14.09
FRZB_T22242A	74.66	69.91	72.74	70.14	82.27	72.18	4.52	-0.23	2.60	10.09
FRZB_G23043A	88.01	91.66	91.15	88.65	88.79	88.89	-0.63	3.01	2.50	-0.10
PRZB_G23415A	83.69	89.48	88.86	84.90	85.30	86.26	-1.21	4.58	3.96	-0.96
FRZB_T23549C	98.30	97.89	97.33	98.56	97.77	97.02	-0.26	-0.67	-1.22	0.75
FRZB_A24791G	50.53	56.92	55.67	56.26	46.12	61.57	-5.73	0.66	-0.59	-15.45
FRZB_C26794G	82.22	81.82	78.24	82.23	80.5	76.66	0.0	-0.41	-3.99	3.84
FRZB_G27014A	96.79	98.69	96.54	95.5	98.62	96.1	1.29	3.2	1.04	2.52

A   Hip Fracture
B   Vertebral Fracture
C   Low BMD
D   Control

Example 3: Individual Genotyping

Because of the significant differences in allele frequency between some osteoporotic case and control groups and between the high and low BMI groups, the individual samples were genotyped to both verify the allele frequency differences and to determine genotype

5   frequencies in these groups. The same allele-specific protocol was used on individual DNA samples to determine their genotypes. The PCR amplifications were performed as described in Example 1. The primers used to genotype the FRZB SNPs were those listed in Table 2. These results were analyzed using Pearson's Chi-Square (or an Exact Test if the number of subjects with one of the genotypes was 10 or less) to determine whether

10   the distribution of genotype frequencies were significantly different between the groups of individuals who had any of the osteoporotic phenotypes and controls and between the groups of individuals with high BMI (a.k.a., obesity) and low BMI.

A significant association between an increased BMI and the FRZB_T2303723C, FRZB_C18679T, FRZB_G19524A and FRZB_T22242A SNPs was demonstrated (p <

15   0.05). A lesser association between an increased BMI and the FRZB_A24791G SNP was also demonstrated (p<0.1). The T allele of T2303723C, the T allele of C18679T, the A allele of G19524A, the A allele of T22242A, and the G allele of A24791G were each demonstrated to be associated with increased BMI (and thus, with an increased risk of obesity). The association is most statistically significant if the effect of each of these

20   alleles is assumed to be recessive.

With respect to osteoporosis, significant associations were demonstrated between increased incidence of vertebral fracture and the FRZB_C26794G and FRZB_G27014A SNPs (p < 0.05). A lesser association between increased incidence of hip fracture and the FRZB_C18679T SNP was also demonstrated (p<0.1), as well as between increased

25   incidence of hip fracture and the FRZB_G19524A SNP (p<0.1). The C allele of C18679T, the G allele of G19524A, the C allele of C26794G, and the G allele of G27014A were each demonstrated to be associated with increased incidence of hip or vertebral fracture (and thus, with an increased risk of osteoporosis). The association is most statistically significant if the effect of each of these alleles is assumed to be recessive.

30   In addition, it is worth noting that after adjustment for weight and age of the patients, the A allele of A24791G is associated with an increased incidence of hip fracture (p<0.1, with the A allele assumed to be recessive). Similarly, using an alternative control group (defined as no fracture since age 50 and highest 5% hip BMD by individual 5-year age

group), the T allele of T22242A is associated with an increased incidence of hip fracture. Again, the association is most statistically significant if the effect of this allele is assumed to be recessive.

Association of FRZB SNPs with Obesity in Women

This example demonstrates the association of FRZB SNPs with obesity in women.

As noted, FRZB genotyping was carried out on women from the Study of Osteoporotic Fractures (SOF), using a genotyping method essentially as described in Example 1. Table 4 lists the genotyping results for the four FRZB SNPs indicated and the numbers and percents of individuals in each BMI category for each genotype.

Table 4: FRZB genotype association with BMI

	C18679T					G19524A			
	BMI	No.	%			BMI	No.	%	Overall Distribution of G19524A P=.0051
T/T	High	18	12.8		A/A	High	14	10.0	
	Low	3	3.7			Low	0	0.0	
T/C	High	55	39.0		A/G	High	50	35.5	
	Low	38	46.3			Low	30	36.6	Model where 19524A is recessive allele P=.0032
C/C	High	68	48.2		G/G	High	77	54.6	
	Low	41	50.0			Low	52	63.4	
Total No. high BMI=141; Total No. low BMI=82									
	T22242A					A24791G			
	BMI	No.	%.			BMI	No.	%	
A/A	High	15	10.7		G/G	High	36	25.5	
	Low	2	2.4			Low	13	15.9	
A/T	High	57	40.4		G/A	High	69	48.9	
	Low	32	39.0			Low	43	52.4	
T/T	High	69	48.9		A/A	High	36	25.5	
	Low	48	58.5			Low	26	31.7	

Statistical analysis, methods and algorithms: Association of FRZB genotypes with obesity was assessed using Pearson's Chi-Square test or, if the number of subjects with one of the genotypes was 10 or less, Fisher's Exact Test. Table 4 shows the p-values

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(probabilities) that the distribution of genotypes at the G19524A SNP between the high
and low BMI groups could have been obtained purely by chance. This is seen to be
highly unlikely. Thus there is a statistical association between G19524A and BMI. The p-
value is even lower if it is assumed that the minor allele, 19524A, exerts its genetic effect
5   in a recessive (two 19524A alleles required) mode.

Association of FRZB With Osteoporosis in SOF Samples

This example demonstrates the association of FRZB SNPs with osteoporosis in SOF
samples.

Table 5 lists the genotyping results for FRZB SNP C26794G and the numbers and
10   percents of individuals in the vertebral fracture and control category for each genotype.

<u>Table 5: FRZB genotype association with vertebral fracture</u>

	C26794G (Arg -> Gly)			
	fracture	No.	%	Overall Distribution
G/G	No	1	0.4	P=.025
	Yes	2	0.8	
G/C	No	52	18.7	
	Yes	29	11.1	
C/C	No	225	80.9	
	Yes	231	88.1	

Total No. vertebral fracture =262; Total No. control =278

Statistical analysis, methods and algorithms: Association of FRZB genotypes with
osteoporosis was assessed using Pearson's Chi-Square test or, if the number of subjects
15   with one of the genotypes was 10 or less, Fisher's Exact Test. Table 5 shows the p-value
(probability) that the distribution of genotypes at C26794G between the vertebral
fracture and control groups could have been obtained purely by chance. This is seen to
be highly unlikely. Thus there is a statistical association between C26794G and vetebral
fracture. C26794G imparts a coding (amino acid) substitution (arginine to glycine)
20   upon the protein sequence of FRZB.

As FRZB is a small component of the complex system of genes associated with obesity and/or osteoporosis (which are thought to be related since bone and fat cells originate from branches along the same developmental pathway), the effect of the FRZB locus is expected to be variable. Other factors, such as eating habits, exercise, diet, general health

5   and the presence of associated diseases, may exert dominating effects which, in some cases, may mask the effect of the FRZB genotypes. Furthermore, because allele frequencies at other loci relevant to weight and bone-related diseases differ between populations and, thus, populations exhibit different risks for such diseases, it is expected that the effect of the FRZB genotype may be of different magnitude in some

10   populations. Although the contribution of the FRZB genotype may, in certain populations, be relatively minor by itself, genotyping at the FRZB locus will contribute information that is, nevertheless, useful for a characterization of an individual's predisposition towards obesity and/or osteoporosis. The FRZB genotype information may be particularly useful when combined with genotype information from other loci

15   and/or clinical tests for obesity and/or osteoporosis, for example.

Although the invention has been disclosed in the context of certain embodiments and examples, it will be understood by those skilled in the art that the invention extends beyond the specifically disclosed embodiments to other alternative embodiments and/or uses and obvious modifications and equivalents thereof. Accordingly, the invention is

20   not intended to be limited by the specific disclosures of preferred embodiments herein, but instead by reference to claims attached hereto. All references cited herein are hereby incorporated by reference in their entireties.

While the foregoing invention has been described in some detail for purposes of clarity and understanding, it will be clear to one skilled in the art from a reading of this

25   disclosure that various changes in form and detail can be made without departing from the true scope of the invention. For example, all the techniques, apparatus and/or compositions described above can be used in various combinations. All publications, patents, patent applications, and/or other documents cited in this application are incorporated by reference in their entirety for all purposes to the same extent as if each

30   individual publication, patent, patent application, and/or other document were individually indicated to be incorporated by reference for all purposes.

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CLAIMS

<u>WHAT IS CLAIMED IS:</u>

1.   A method for determining an individual's risk for obesity, the method
comprising: detecting presence of at least one obesity-related polymorphism in a
frizzled-related protein (FRZB) gene in a nucleic acid sample of the individual,
wherein the presence of said at least one polymorphism provides an indication of
the individual's risk for obesity.

2.   The method of claim 1, wherein the individual's risk for obesity is an increased
risk as compared to an individual without the at least one polymorphism.

3.   The method of claim 1, wherein the individual's risk for obesity is a decreased
risk as compared to an individual without the at least one polymorphism.

4.   The method of claim 1, wherein the at least one polymorphism comprises a
predisposing or protective polymorphism in the FRZB gene.

5.   The method of claim 1, wherein the nucleic acid sample comprises DNA or
RNA.

6.   The method of claim 1, wherein the at least one polymorphism comprises at least
one single nucleotide polymorphism (SNP).

7.   The method of claim 6, wherein the at least one polymorphism is selected from
the group consisting of: T allele of T2303723C, C allele of T2303723C, C allele of
C18679T, T allele of C18679T, G allele of G19524A, A allele of G19524A, T allele
of T22242A, A allele of T22242A, A allele of A24791G, and G allele of A24791G.

8.   The method of claim 1, wherein the at least one polymorphism comprises two or
more polymorphisms.

9.   The method of claim 8, wherein at least one of the two or more polymorphisms
is selected from the group consisting of: T allele of T2303723C, C allele of
T2303723C, C allele of C18679T, T allele of C18679T, G allele of G19524A, A

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allele of G19524A, T allele of T22242A, A allele of T22242A, A allele of A24791G, and G allele of A24791G.

10.   The method of claim 8, wherein the two or more polymorphisms are selected from the group consisting of: T allele of T2303723C, C allele of T2303723C, C allele of C18679T, T allele of C18679T, G allele of G19524A, A allele of G19524A, T allele of T22242A, A allele of T22242A, A allele of A24791G, and G allele of A24791G.

11.   The method of claim 1, wherein the at least one polymorphism is detected by sequencing.

12.   The method of claim 1, wherein the at least one polymorphism is detected by amplification.

13.   The method of claim 12, wherein the amplification comprises a polymerase chain reaction or a ligase chain reaction.

14.   The method of claim 1, wherein the detecting comprises:

-       contacting the nucleic acid sample with at least one sequence-specific oligonucleotide under conditions that allow binding of said at least one oligonucleotide to the nucleic acid sample, wherein the at least one sequence-specific oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the at least one obesity-related polymorphism; and,

-       detecting the hybridization of the at least one oligonucleotide to the nucleic acid sample.

15.   The method of claim 1, wherein the detecting comprises:

-       amplifying the nucleic acid sample, thereby providing an amplified nucleic acid sample;

-       contacting the amplified nucleic acid sample with at least one sequence-specific oligonucleotide under conditions that allow binding of the oligonucleotide to the amplified nucleic acid sample,

wherein the at least one sequence-specific oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the at least one obesity-related polymorphism; and,

- detecting the hybridization of the at least one sequence-specific oligonucleotide to the amplified nucleic acid sample.

16. The method of claim 1, wherein detecting the presence of the at least one obesity-related polymorphism comprises qualitatively detecting the presence of the at least one obesity-related polymorphism.

17. The method of claim 1, wherein detecting the presence of the at least one obesity-related polymorphism comprises quantitatively detecting the presence of the at least one obesity-related polymorphism.

18. The method of claim 1, wherein the presence of the polymorphism inherited from one of the individual's parents provides an indication of the individual's risk for obesity, or wherein the presence of the polymorphism inherited from both of the individual's parents provides an indication of the individual's risk for obesity.

19. The method of claim 1, comprising performing at least one clinical test for obesity.

20. The method of claim 19, wherein performing the at least one clinical test for obesity comprises determining a body mass index (BMI) of the individual.

21. A method for determining an individual's risk for osteoporosis, the method comprising: detecting presence of at least one osteoporosis-related polymorphism in an FRZB gene in a nucleic acid sample of the individual, wherein the presence of said at least one polymorphism provides an indication of the individual's risk for osteoporosis.

22. The method of claim 21, wherein the individual's risk for osteoporosis is an increased risk as compared to an individual without the at least one polymorphism.

23.   The method of claim 21, wherein the individual's risk for osteoporosis is a decreased risk as compared to an individual without the at least one polymorphism.

24.   The method of claim 21, wherein the at least one polymorphism comprises a predisposing or protective polymorphism in the FRZB gene.

25.   The method of claim 21, wherein the nucleic acid sample comprises DNA or RNA.

26.   The method of claim 21, wherein the at least one polymorphism comprises at least one single nucleotide polymorphism (SNP).

27.   The method of claim 26, wherein the at least one polymorphism is selected from the group consisting of: C allele of C18679T, T allele of C18679T, G allele of G19524A, A allele of G19524A, A allele of A24791G, G allele of A24791G, C allele of C26794G, G allele of C26794G, G allele of G27014A, and A allele of G27014A.

28.   The method of claim 21, wherein the at least one polymorphism comprises two or more polymorphisms.

29.   The method of claim 28, wherein at least one of the two or more polymorphisms is selected from the group consisting of: C allele of C18679T, T allele of C18679T, G allele of G19524A, A allele of G19524A, A allele of A24791G, G allele of A24791G, C allele of C26794G, G allele of C26794G, G allele of G27014A, and A allele of G27014A.

30.   The method of claim 28, wherein the two or more polymorphisms are selected from the group consisting of: C allele of C18679T, T allele of C18679T, G allele of G19524A, A allele of G19524A, A allele of A24791G, G allele of A24791G, C allele of C26794G, G allele of C26794G, G allele of G27014A, and A allele of G27014A.

31.   The method of claim 21, wherein the at least one polymorphism is detected by sequencing.

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32.   The method of claim 21, wherein the at least one polymorphism is detected by amplification.

33.   The method of claim 32, wherein the amplification comprises a polymerase chain reaction or a ligase chain reaction.

5   34.   The method of claim 21, wherein the detecting comprises:

-   contacting the nucleic acid sample with at least one sequence-specific oligonucleotide under conditions that allow binding of the oligonucleotide to the nucleic acid sample, wherein the at least one sequence-specific oligonucleotide hybridizes under stringent

10   conditions to a region of the FRZB gene comprising the at least one osteoporosis-related polymorphism; and,

-   detecting the hybridization of the at least one sequence-specific oligonucleotide to the nucleic acid sample.

35.   The method of claim 21, wherein the detecting comprises:

15   -   amplifying the nucleic acid sample, thereby providing an amplified nucleic acid sample;

-   contacting the amplified nucleic acid sample with at least one sequence-specific oligonucleotide under conditions that allow binding of the oligonucleotide to the amplified nucleic acid sample,

20   wherein the at least one sequence-specific oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the at least one osteoporosis-related polymorphism; and,

-   detecting the hybridization of the at least one sequence-specific oligonucleotide to the amplified nucleic acid sample.

25   36.   The method of claim 21, wherein detecting the presence of the at least one osteoporosis-related polymorphism comprises qualitatively detecting the presence of the at least one osteoporosis-related polymorphism.

37.   The method of claim 21, wherein detecting the presence of the at least one
        osteoporosis-related polymorphism comprises quantitatively detecting the
        presence of the at least one osteoporosis-related polymorphism.

38.   The method of claim 21, wherein the presence of the polymorphism inherited
        from one of the individual's parents provides an indication of the individual's
        risk for osteoporosis, or wherein the presence of the polymorphism inherited
        from both of the individual's parents provides an indication of the individual's
        risk for osteoporosis.

39.   The method of claim 21, comprising performing at least one clinical test for
        osteoporosis.

40.   The method of claim 39, wherein the at least one clinical test for osteoporosis
        comprises a bone-turnover assay or a bone scan.

41.   A method for determining an individual's risk for obesity and/or osteoporosis,
        the method comprising: determining the individual's genotype at one or more
        polymorphic sites in an FRZB gene, wherein a first genotype at the one or more
        polymorphic sites is statistically associated with an increased risk for obesity
        and/or osteoporosis as compared to a second genotype at the one or more
        polymorphic sites.

42.   The method of claim 41, wherein at least one of the one or more polymorphic
        sites consists of a single nucleotide position.

43.   The method of claim 42, wherein the individual's genotype is determined at a
        plurality of polymorphic sites in the FRZB gene, and wherein each of the
        polymorphic sites consists of a single nucleotide position.

44.   The method of claim 41, wherein at least one of the one or more polymorphic
        sites is selected from the group consisting of: nucleotide position 2628,
        nucleotide position 18679, nucleotide position 19524, nucleotide position 22242,
        nucleotide position 24791, nucleotide position 26794, and nucleotide position
        27014 of SEQ ID NO:1.

45.   The method of claim 41, wherein the first genotype is statistically associated with
        an increased risk for obesity as compared to the second genotype; and wherein
        the first genotype comprises two T alleles and the second genotype two C alleles
        or one T allele and one C allele of SNP T2303723C, the first genotype comprises
        two T alleles and the second genotype two C alleles or one T allele and one C
        allele of SNP C18679T, the first genotype comprises two A alleles and the second
        genotype two G alleles or one A allele and one G allele of SNP G19524A, the first
        genotype comprises two A alleles and the second genotype two T alleles or one A
        allele and one T allele of SNP T22242A, and/or the first genotype comprises two
        G alleles and the second genotype two A alleles or one G allele and one A allele of
        SNP A24791G.

46.   The method of claim 41, wherein the first genotype is statistically associated with
        an increased risk for osteoporosis as compared to the second genotype; and
        wherein the first genotype comprises two C alleles and the second genotype two
        T alleles or one T allele and one C allele of SNP C18679T, the first genotype
        comprises two G alleles and the second genotype two A alleles or one A allele and
        one G allele of SNP G19524A, the first genotype comprises two A alleles and the
        second genotype two G alleles or one G allele and one A allele of SNP A24791G,
        the first genotype comprises two C alleles and the second genotype two G alleles
        or one C allele and one G allele of SNP C26794G, and/or the first genotype
        comprises two G alleles and the second genotype two A alleles or one G allele and
        one A allele of SNP G27014A.

47.   The method of claim 41, wherein determining the individual's genotype
        comprises obtaining a nucleic acid sample from the individual.

48.   The method of claim 47, wherein determining the individual's genotype
        comprises amplifying at least a portion of the FRZB gene from the nucleic acid
        sample, the portion comprising at least one of the one or more polymorphic
        sites.

49.   The method of claim 47, wherein determining the individual's genotype
        comprises performing an allele-specific amplification or an allele-specific
        extension reaction.

50.   The method of claim 47, wherein determining the individual's genotype comprises sequencing at least a portion of the FRZB gene from the nucleic acid sample, the portion comprising at least one of the one or more polymorphic sites.

·5   51.   The method of claim 47, wherein at least one of the one or more polymorphic sites consists of a single nucleotide position, the method comprising:

-    contacting the nucleic acid sample with at least one sequence-specific oligonucleotide under stringent conditions, wherein the oligonucleotide hybridizes under the stringent conditions to the
10   nucleic acid sample when a first nucleotide occupies the nucleotide position defining the polymorphic site but not when a second nucleotide occupies the nucleotide position, and

-    detecting hybridization of the oligonucleotide to the nucleic acid sample.

15   52.   A kit for detecting presence of a first predisposing or protective polymorphism in an FRZB gene in a nucleic acid sample of an individual whose risk for osteoporosis and/or obesity is being assessed, the kit comprising: one or more first oligonucleotides capable of detecting the first polymorphism, and instructions for detecting the first polymorphism with the one or more first
20   oligonucleotides and for correlating said detection to the individual's risk for osteoporosis and/or obesity, packaged in one or more containers.

53.   The kit of claim 52, wherein the first polymorphism is a single nucleotide polymorphism.

54.   The kit of claim 53, wherein the first polymorphism is selected from the group
25   consisting of: T allele of T2303723C, C allele of T2303723C, C allele of C18679T, T allele of C18679T, G allele of G19524A, A allele of G19524A, T allele of T22242A, A allele of T22242A, A allele of A24791G, G allele of A24791G, C allele of C26794G, G allele of C26794G, G allele of G27014A, and A allele of G27014A.

55.   The kit of claim 52, wherein the one or more first oligonucleotides comprise at
30   least one probe.

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56.   The kit of claim 52, wherein the first oligonucleotide hybridizes under stringent conditions to a region of the FRZB gene comprising the first polymorphism.

57.   The kit of claim 56, wherein the first polymorphism is a first single nucleotide polymorphism comprising a first nucleotide at a first nucleotide position, and wherein, under stringent conditions, the first oligonucleotide hybridizes to a region of the FRZB gene comprising the first single nucleotide polymorphism with a signal to noise ratio that is at least 2x that at which the first oligonucleotide hybridizes to the region of the FRZB gene comprising a second nucleotide at the first nucleotide position.

58.   The kit of claim 56, wherein the first oligonucleotide is fully complementary to the region of the FRZB gene comprising the first polymorphism.

59.   The kit of claim 56, wherein the first oligonucleotide comprises at least about 10 contiguous nucleotides complementary to the FRZB gene.

60.   The kit of claim 52, wherein the one or more first oligonucleotides comprise a label.

61.   The kit of claim 60, wherein the label comprises an isotopic, fluorescent, fluorogenic or colorimetric label.

62.   The kit of claim 60, comprising a reagent, wherein the reagent detects the label.

63.   The kit of claim 52, wherein the one or more first oligonucleotides comprise one or more primers.

64.   The kit of claim 63, wherein the first polymorphism is a first single nucleotide polymorphism comprising a first nucleotide at a first nucleotide position, and wherein the 3' nucleotide of one of the one or more first oligonucleotides is complementary to the first nucleotide.

65.   The kit of claim 52, wherein the one or more first oligonucleotides comprise amplification primers, wherein the amplification primers amplify a nucleic acid sequence comprising the first polymorphism.

66.   The kit of claim 52, wherein the one or more first oligonucleotides comprise
       sequencing primers, wherein the sequencing primers flank the first
       polymorphism.

67.   The kit of claim 52, wherein the one or more first oligonucleotides are
5   immobilized on a substrate.

68.   The kit of claim 52, comprising one or more second oligonucleotides capable of
       detecting a second polymorphism.

69.   An array for detecting presence of one or more predisposing and/or protective
       polymorphisms in an FRZB gene in a nucleic acid sample of an individual whose
10   risk for osteoporosis and/or obesity is being assessed, the array comprising:

       -    a plurality of oligonucleotides, each of which oligonucleotides
            hybridizes to a region of the FRZB gene comprising at least one of the
            polymorphisms, whereby said hybridization detects the presence of
            the polymorphism, whereby said detection provides an indication of
15   the individual's risk for osteoporosis and/or obesity; and,

       -    a substrate on which the plurality of oligonucleotides are
            immobilized.

70.   The array of claim 69, wherein the one or more polymorphisms comprise one or
       more single nucleotide polymorphisms.

20 71. The array of claim 70, wherein at least one of the one or more polymorphisms is
       selected from the group consisting of: T allele of T2303723C, C allele of
       T2303723C, C allele of C18679T, T allele of C18679T, G allele of G19524A, A
       allele of G19524A, T allele of T22242A, A allele of T22242A, A allele of A24791G,
       G allele of A24791G, C allele of C26794G, G allele of C26794G, G allele of
25   G27014A, and A allele of G27014A.

72.   The array of claim 70, wherein, under stringent conditions, each of the
       oligonucleotides hybridizes to a region of the FRZB gene comprising one of the
       single nucleotide polymorphisms with a signal to noise ratio that is at least 2x

that at which the oligonucleotide hybridizes to a region of the FRZB gene comprising any of the remaining single nucleotide polymorphisms.

73. The array of claim 70, wherein each of the oligonucleotides hybridizes to a distinct single nucleotide polymorphism.

74. The array of claim 69, wherein each of the plurality of oligonucleotides is immobilized at a known, pre-determined position on the substrate.

75. The array of claim 69, wherein each of the plurality of oligonucleotides is fully complementary to a region of the FRZB gene comprising one of the polymorphisms.

76. The array of claim 69, wherein each of the plurality of oligonucleotides comprises at least about 10 contiguous nucleotides complementary to the FRZB gene.

77. The array of claim 69, wherein each of the plurality of oligonucleotides comprises a label.

78. A system, comprising: the array of claim 69 and system instructions that correlate said detection of the presence of one or more predisposing or protective polymorphisms to the individual's risk for osteoporosis and/or obesity.

- 1/1 -

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FIG. 1

SEQUENCE LISTING

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INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7   C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7   C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used) —

EPO-Internal, WPI Data, PAJ, MEDLINE, BIOSIS, EMBASE, CHEM ABS Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
X	CIESIELSKI C.J. ET AL.,: "single nucleotide polymorphism in FZRB in hereditary chondrolysis syndrome" ARTHRITIS AND RHEUMATISM, vol. 46, no. 9, September 2002 (2002-09), page S274, XP009036551 abstract	21-78
A	LOUGHLIN J. ET AL.,: "refined linkage mapping of a hip osteoarthritis susceptibility locus on chromosome 2q" RHEUMATOLOGY, vol. 41, no. 8, August 2002 (2002-08), pages 955-956, XP002298045 the whole document	1-78
	-/--	

[X] Further documents are listed in the continuation of box C.   [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 September 2004	11/10/2004

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mueller, F

Form PCT/ISA/210 (second sheet) (January 2004)

page 1 of 2

INTERNATIONAL SEARCH REPORT

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	LOUGHLIN J. ET AL.,: "functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis" PNAS, USA, vol. 101, no. 26, 29 June 2004 (2004-06-29), pages 9757-9762, XP002298046	

INTERNATIONAL SEARCH REPORT

Box II   Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III   Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

   see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest   ☐ The additional search fees were accompanied by the applicant's protest.

                               ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM   PCT/ISA/ 210

This International Searching Authority found multiple (groups of)
inventions in this international application, as follows:

    1. claims: 1-20(complete), 41-78 (partial)

        polymorphisms in the FRZB gene related to obesity
            ---

    2. claims: 21-40 (complete), 42-78 (partial)

        polymorphisms in the FRZB gene related to osteoporosis
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